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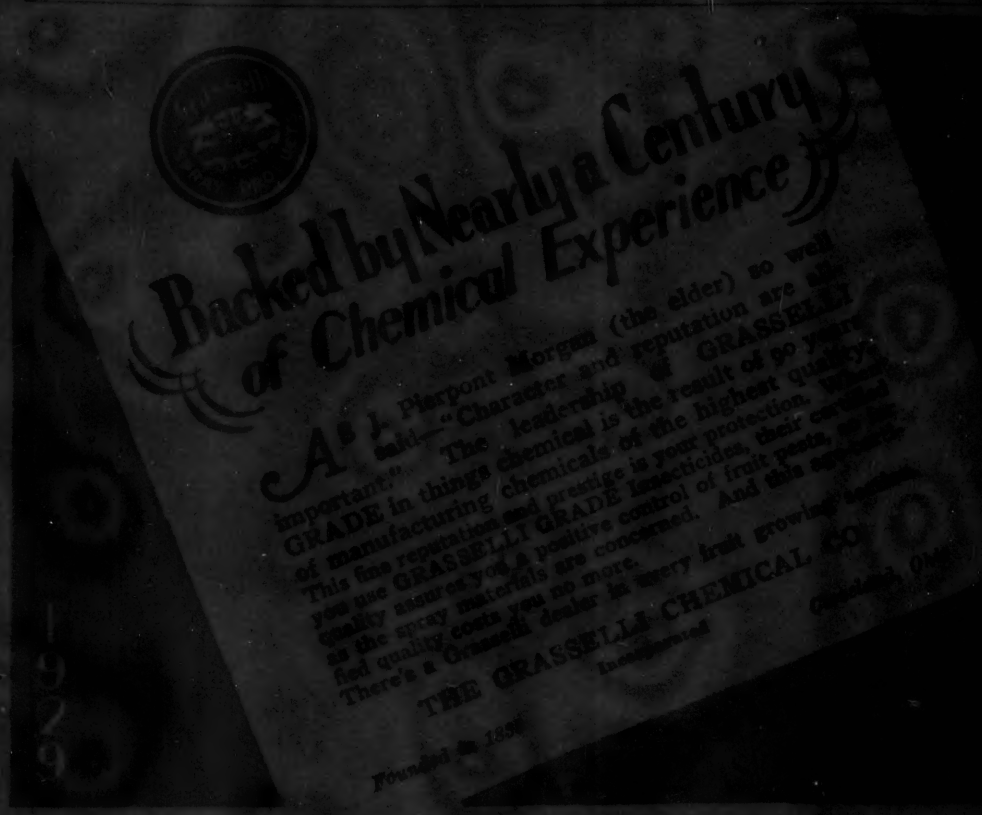
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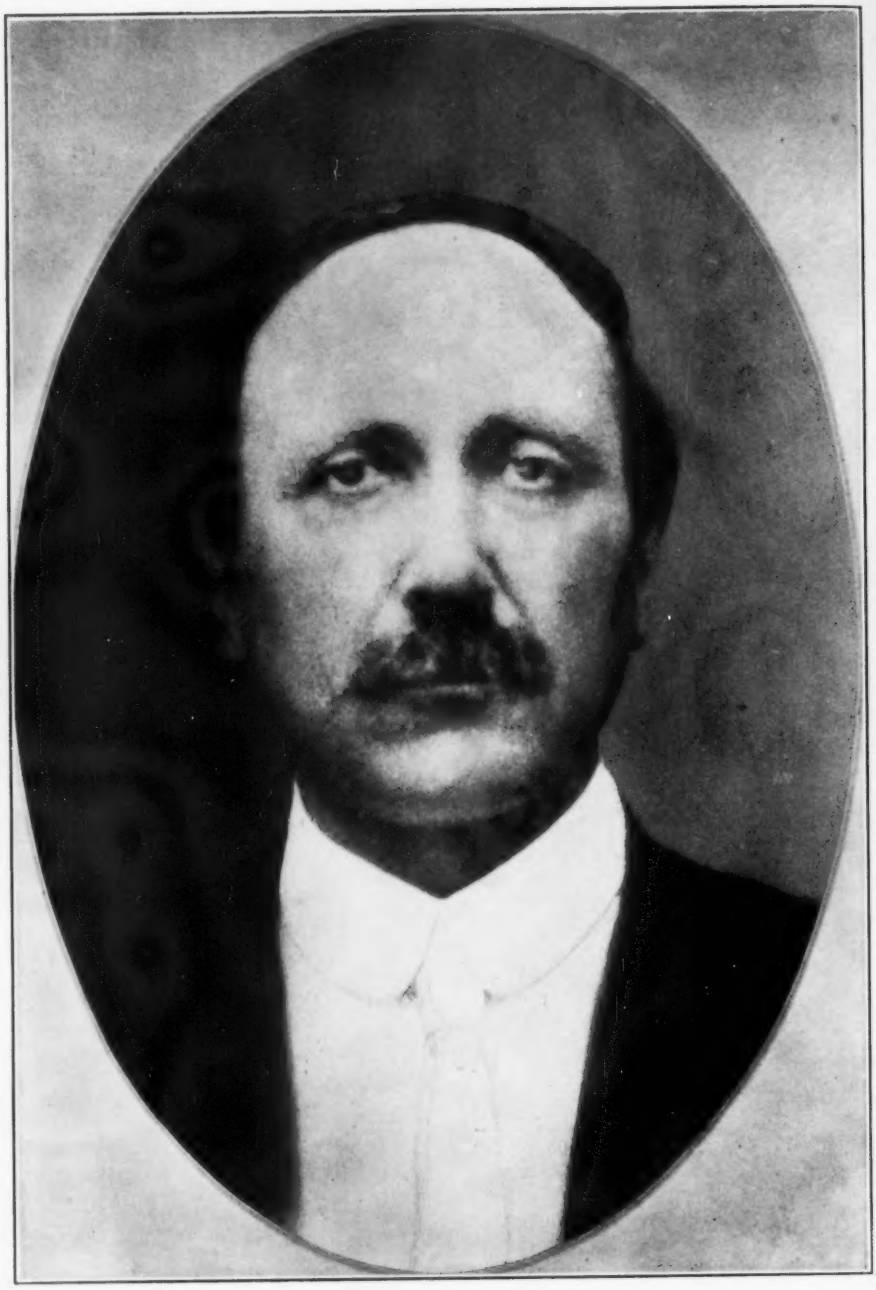

MARK ALFRED CARLETON

PHYTOPATHOLOGY

VOLUME 19 NUMBER 4

APRIL, 1929

MARK ALFRED CARLETON
1866–1925

E. M. FREEMAN

The death of Mark Alfred Carleton, in Paita, Peru, on April 26, 1925, marked the passage of a great pioneer in more than one field of botanical and agricultural science. Carleton's years were singularly eventful. He made several excursions into foreign lands, his scientific contributions covered a wide range of subjects, and his scientific activities developed important contacts with industrial and agricultural enterprises. He was primarily a man of action and packed into the fifty-nine years of his life an amazing record of achievements. Born (1866) near Jerusalem, Ohio, he passed his boyhood in the stern environment of the pioneer agriculture of that day, which left on his character its impress of ruggedness and a lifelong appreciation and sympathy for the farmer of the great middle west. This attitude was further accentuated by his college and scientific training in midwestern institutions of higher education.

The detailed story of Carleton's life has been presented in several biographical sketches (see bibliography) and need not be repeated here. The unfortunate events of the last years of his administrative career as Cerealist of the United States Department of Agriculture and the distressing struggles of the succeeding years, which ended in his death in far-off Peru, have aroused partisan sympathies which make, perhaps, a fair and just appreciation of Carleton's place in history difficult at this time.

Carleton's serious scientific career began in Kansas with his work on the diseases of cereals, and especially on the rusts of small grains. From 1893 to 1905 he published numerous contributions on the grain rusts of the United States. The classic work of Jakob Eriksson in Sweden had demonstrated the complexity of physiologic-form relations in European grain rusts. Carleton laid a firm basis for a similar demonstration of the physiologic-form relation in American rusts of cereals. It is chiefly upon these contributions that Carleton's achievement as a plant pathologist must rest. Although this work was of a pioneering nature, it was comprehensive and exhaustive, has stood the test of time, and will stand as one of the significant contributions to American phytopathology.

To a man like Carleton, scientific investigations required early translation into agricultural practice. The need for wheats resistant to rust and adapted to the Great-Plains region became to him a dominant problem. The rapid expansion of the personnel of the Office of Cereal Investigations from about 1905 to 1910 gave him the opportunity of attempting to meet this and many other problems of small-grain improvement. From this point on, his interests and activities became chiefly those of the agronomist. Probably in this field lie Carleton's greatest contributions to science and agriculture. His explorations and field observations and the extensive introductions and field-plot experiments of the last ten years of his administration of the Office of Cerealist made some of the really great contributions to American agronomy. The introduction of rust-resistant and drought-resistant durums and of winter-hardy Turkey wheats are only two of the many outstanding achievements of this period of his work. The office of which he was chief introduced hundreds of varieties of small grains, tested them in all of the potential grain-growing areas of the United States, inaugurated field studies covering wide ranges of ecologic relations, studied small grains from almost every scientific and practical angle, and undertook basic and applied studies on the breeding of cereal crops.

Carleton's work as general manager for the Pennsylvania Chestnut Blight Commission in 1913–14, one of the few excursions away from the field of agronomy, and those contributions subsequent to his resignation as Cerealist of the United States Department of Agriculture in 1918, chiefly in the field of tropical plant pathology, constitute his minor scientific achievements. The latter were made under stress of financial difficulties, in an unfriendly tropical environment, and in a field foreign to his earlier experience.

Of Carleton as a scientist, the outstanding characteristics were a remarkably keen faculty for field observations and an equally remarkable ability to retain such facts. He was independent and bold in his thinking, and slow to recede from a position once taken. He had a wide knowledge of the literature of his field and was particularly rich in his knowledge of field conditions. He had a large research curiosity but was not satisfied with scientific results until applied in practice. The last ten years of his administration of the Office of Cerealist was a tremendous growth and expansion in the office and field personnel and activities. Carleton was not, by nature, an administrator. He was rather the field investigator. Unfortunate financial ventures and serious illness in his family, in addition to the demands of a rapidly growing and complex organization—demands that were irksome in contrast to his natural inclination for personal field study—gradually brought on difficult, if not impossible, situations. His resigna-

tion from the Office of Cerealist in 1918 was the culmination of one of the not infrequent tragedies in the history of science. Ambition and enthusiasm of man may build a machine that outgrows his power to direct and control.

The administrative rock on which Carleton's career was wrecked is of historical interest only as the agency which terminated the most important phase of his scientific contributions. It can in no wise detract from the real and significant contributions which he made to agronomy, to plant pathology, and to practical agriculture, as well as those of minor importance which he made in other branches of plant science. He was one of the greatest pioneers in American agronomy and deserves a high rank among the pioneer plant pathologists of this country.

As a man, Carleton had a personality that held many staunch friends and developed numerous enemies. To those who knew him intimately, he exhibited a lovable and friendly personality. He was simple and unostentatious in manner but was sometimes lacking in tact. He was firm in his convictions but was not blind either to argument or contrary evidence. He was an untiring and persistent worker. His large size and slow physical actions concealed an unsuspected alertness of mind. He was a bold dreamer in scientific theories. In practical affairs his optimism frequently got the better of his judgment. His dominant characteristic was an untiring and persistent will to work and an uncompromising and unyielding persistence in the pursuit of his cherished projects.

UNIVERSITY FARM,
SAINT PAUL, MINN.

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EXPERIMENTS WITH REPEATING PINE RUSTS

E. P. MEINECKE

FOUR FIGURES

INTRODUCTION

In two former papers (8 and 9) the writer has discussed his experiments with repeating pine rusts. In 1916 he reported the first successful transfer of the aecial stage from pine to pine, under controlled conditions, with omission of an alternate host and of the uredinial and telial stages. At the same time Haack (4) had been conducting a series of independent experiments with *Peridermium pini* in Germany, the results of which reached this country late on account of the war. Unfortunately Haack had performed all his inoculations in the open, in the infected forest, and had selected, for his experiments, trees already heavily infected with *P. pini*. His deductions, namely that *P. pini* can be transferred directly from pine to pine through aeciospores, were therefore not valid. No alternate host for *P. pini* is known. Haack's work found a first reaction in a review by Fischer (3) who reported it with critical reservations. Haack's paper was discussed by Tubeuf (11). He emphasizes the inconclusive character of Haack's results and reports (11, p. 274) unsuccessful inoculations of his own with the same rust on *Pinus silvestris* and *P. montana* (1915 and 1916). The writer's positive results, obtained with a rigidly controlled technique, would in themselves have lent strong probability to Haack's findings, had not Klebahn (7), unaware of the fact that the problem had been definitely solved, taken up Haack's work and repeated it under controlled conditions. He proved that the aeciospores of *Peridermium pini* are capable of infecting *Pinus silvestris* directly. *Peridermium pini* is, then, the second example of this exception to the general rule of heteroecism in the genus Cronartium, the Peridermium on *Pinus radiata* investigated by the writer being the first. A short paper by Tubeuf (12) refers again to Haack, who in the meantime had fallen in the war, and accepts Klebahn's conclusions.

Owing to the war blockade neither Haack, Klebahn nor Tubeuf knew of the work of the writer. Tubeuf's papers finally arrived in this country some time after the war was over. The writer's second publication on the subject (8) gave the results of a series of experiments in corroboration of his first paper, and reported similar success with a third Peridermium (*Cronartium harknessii*, nov. comb.).

The writer's work dealt with a Peridermium on *Pinus radiata* and a few other pines of the coastal region and with a Peridermium confined to the

pines of the interior mountain regions of California, such as *Pinus contorta, P. ponderosa* and *P. sabiniana.* The Peridermium on *Pinus radiata* was first considered to be *Peridermium harknessii* Moore. On the strength of inoculation experiments on oaks reported by Hedgcock (10) the rust was later classed as *Peridermium cerebrum* Peck (*Cronartium cerebrum* (Peck) Hedg. and Long), particularly since uredinial and telial forms are common on oaks in the *Pinus radiata-Quercus agrifolia* association near the seashore in California. Hedgcock's results had been meager and in a recent personal communication to the writer Hedgcock himself doubts the validity of the experiment. Without wishing to discuss at this time the question of the true identity of the rust, the writer proposes to refer to this rust in the following no longer as *Peridermium cerebrum* Peck but *Peridermium cerebroides* (*?*). It seems probable that we have to do with a new form for which a valid name is lacking. It is not impossible that the rust may have lost altogether its faculty to infect any of the plants now available within the range of its aecial hosts. The same seems to be true of *Peridermium pini.* The other Californian pine gall rust, apparently confined to mountain pines, is designated by the writer as *Peridermium harknessii* (*Cronartium harknessii,* nov. comb). Arthur (1) seems inclined to place this rust under *Cronartium coleosporioides* (Diet. and Holway) Arth. and to maintain the presence of *Cronartium cerebrum* (Peck) Hedgcock and Long on the Pacific Coast. The writer now believes that the common gall-forming Peridermium on *Pinus radiata* is not *Peridermium cerebrum.* He still believes that *Peridermium stalactiforme* is a synonym of *P. filamentosum. Cronartium coleosporioides,* in the writer's opinion, comprises one or two, perhaps even three non-gall-forming Peridermia. In order to avoid further confusion he will refer in the following to the rust which causes galls on *Pinus contorta, P. ponderosa* and other pines of the mountain regions of California as *Cronartium harknessii.*

INOCULATION EXPERIMENTS

All the experiments reported in the following were carried out at the Feather River Experiment Station of the United States Forest Service near Quincy, Plumas Co., Calif. Quincy lies at an elevation of 3600 feet in the northern extension of the Sierra Nevada. The Station is located in a forest composed of *Pinus ponderosa, Pinus lambertiana, Pseudotsuga taxifolia, Abies concolor* and *Libocedrus decurrens,* an association in which *Pinus ponderosa* represents the character tree. The location is several hundred miles distant from the nearest host of *Peridermium cerebroides* (*?*). *Peridermium harknessii* is exceedingly rare. In the course of ten years only a single gall has been observed, in spite of consistent and careful search. This

gall, found in 1918 on a young Western yellow pine, was immediately destroyed. *Castilleia miniata,* its most common alternate host, is at home in the vicinity, but the rust has never been found on it. A greenhouse and a nursery offer excellent conditions for clean and controlled work. The methods of inoculation used were either that of introducing aeciospores into tangential slits of the type described previously (8, p. 286) or that of showering the plants with aeciospores. In the latter case the plants were first sprayed with water and the spores were either dusted over the plants or sprayed on, suspended in water. After inoculation the plants were maintained for several days in a moist atmosphere of even temperature by the use of bell jars or portable inoculation chambers made of panes of celluloid and cheesecloth nailed on light wooden frames.

PERIDERMIUM HARKNESSII

The identity of the material used was proved by the successful inoculation of plants of *Castilleia* (*miniata?*) with aeciospores from typical galls on *Pinus contorta* and *P. sabiniana.*

Slit Method. Table 1 gives the results of inoculations made in the greenhouse by the introduction of aeciospores into tangential slits through the epidermis and cortex layers of the main stem of young pines. Of a total of 113 inoculated slits 79, or 70 per cent, developed definite galls. The

TABLE 1.—*Infections by the slit method. Peridermium harknessii*

	Slits	Positive	Negative
1. *P. contorta–P. contorta*	16	11	5
2. *P. contorta–P. radiata*	32	32	—
3. *P. sabiniana–P. radiata*	12	9	3
4. *P. sabiniana–P. jeffreyi*	12	9	3
5. *P. sabiniana–P. coulteri*	8	5	3
6. *P. (contorta) contorta–P. contorta*	10	7	3
7. *P. (jeffreyi) contorta–P. jeffreyi*	15	4	11
8. *P. (jeffreyi) contorta–P. radiata*	8	2	6
	113	79	34

last four inoculations are of particular interest. For No. 5 (*Pinus sabiniana-P. coulteri*) small seedlings of about 2″ or 5 cm. in height were used. The seeds had been sown in the greenhouse in November, 1919, and the inoculation took place in April, 1920. The extreme tenderness of the thin young stems makes the incision as well as the insertion of spore material a delicate performance. The positive results obtained in over 62 per cent of the slits show that even very young tissues are susceptible to infec-

tion. Each plant could receive not more than one slit. Two years after the inoculation one of the plants had attained a height of 16 cm. and bore a gall measuring 7.5×1.2 cm., accompanied by a well-developed witches'-broom. The experiment adds *Pinus coulteri* to the list of host plants artificially infected with *Peridermium harknessii*. Nos. 6, 7 and 8 were made with pedigreed material, the result of former inoculations by direct transfer of aeciospores, so that the resulting galls represent the third generation under control. While No. 6 (*Pinus* (*contorta*) *contorta-contorta*) shows 70 per cent success, the No. 7 and 8 (*P.* (*jeffreyi*) *contorta-jeffreyi* and *P.* (*jeffreyi*) *contorta-radiata*) were successful only in 26 and 25 per cent of the inoculations. Moreover No. 6 developed very slowly.

All check plants remained free from infection.

Spore Showers. In the first paper of this series the writer had expressed the opinion that the infection of pines with aeciospores took place largely through wounds, caused probably by insects. In order to verify or disprove this assumption he attempted to inoculate young pines by showering them with aeciospores. The plants were apparently free from insects and had been carefully handled in order to avoid accidental wounding. The method is not new in its application to repeating rusts on pines. Klebahn (7, p. 200, 201) had dusted young specimens of *Pinus silvestris* with aeciospores of *Peridermium pini* and had obtained galls on unwounded plants, and Tubeuf (11, p. 274) reports similar but unsuccessful experiments.

The writer's inoculations by the method of showering the plants with aeciospores were carried out partly in the greenhouse, and partly in the nursery. In the latter case the plants were protected and isolated for several weeks with bell-jars or portable inoculation chambers. In spite of the fact that the region of the Experiment Station is, apparently at least, practically free from *Peridermium harknessii* the results of the nursery experiments would not be valid, had not the fact of direct aecial transfer been abundantly shown by greenhouse experiments and by the fact that of the many hundreds of plants in the nursery no galls appeared except on inoculated plants. As in the greenhouse, all galls showing the beginning of sporulation were either at once removed or carefully wrapped in cheesecloth to prevent an accidental transmission of spores. The results obtained in the nursery have therefore the value of corroborative data. Of 14 pines showered in the greenhouse 11, or 78.5 per cent, became infected and developed 47 galls or an average of 4 galls per plant. Of 18 pines showered in the nursery 8 plants, or 44 per cent, developed 27 galls, averaging a little over 3 galls per plant.

Peridermium harknessii is known to go to *Castilleia sp.*, an alternate host. One could think of a differentiation of the rust species into strains,

one of which had retained the faculty of heteroecism while the other had lost it. That this explanation has no foundation was proved by inoculation of *Castilleia* (*miniata?*) with the same aeciospore material which was used for Nos. 1, 3, 4 and 5 of table 1. All of these inoculations, made in the usual manner in the greenhouse, were successful, reaching the uredinial, and in one plant, the telial stage. The check plants remained uninfected.

Peridermium cerebroides (*?*)

The inoculations with this species were made in the greenhouse, under the usual precautions. Their main objective was to ascertain whether this Peridermium required wounding of the host as a condition for entrance. Four plants of *Pinus radiata* were sprayed with water. The spore dust of *Peridermium cerebroides* (*?*) from *Pinus radiata* was then applied with a sterile brush. Of the four plants three became infected and produced 12 galls or an average of 4 galls per plant. Fig. 1 illustrates the location

FIG. 1. Typical galls of *Peridermium cerebroides* (*?*) developing on *Pinus radiata* as a result of showering the plants with aeciospores.

and type of galls produced. The large plant to the right has escaped infection. One of the infected plants died and was removed.

Peridermium occidentale, P. filamentosum, P. pyriforme.

All attempts to transmit *Peridermium occidentale, P. filamentosum* or *P. pyriforme* from one pine to another by the use of aeciospores failed to

produce results. All plants were kept under observation for at least two years. The details are given in the following tabulation:

Peridermium occidentale.

Slit method: *Pinus edulis-P. monophylla* 23 slits inoculated.
" *monophylla-P. monophylla* 9 slits inoculated.
Spore shower: " *monophylla-P. monophylla* 6 plants inoculated.

On three of the latter plants necrotic spots appeared without developing into more significant stages. These experiments should be repeated.

Peridermium filamentosum.

Inoculations were made from *Pinus jeffreyi* to *P. jeffreyi, P. contorta* to *P. contorta* and *P. ponderosa* to *P. ponderosa.* Two plants were showered with spores and 59 slits were inoculated. All experiments were unsuccessful.

Peridermium pyriforme.

In the attempt to transmit the rust directly from *Pinus ponderosa* to *P. ponderosa* 28 slits and incisions were inoculated and three plants were showered with aeciospores. The experiments remained without results after being under observation for six years.

INCUBATION PERIOD

The length of the incubation period varies greatly. Undoubtedly the condition of the inoculum and of the plant inoculated as well as the climatic conditions under which the infected plant is kept are important factors. The time of inoculation, the proper synchronization of the physiological state of the inoculum with that of the new host must surely have their influence upon the establishment of the rust within the plant. But of all factors which determine the length of the incubation period the relative susceptibility of the species and of the individual plant is perhaps the most weighty. Of all these relationships very little is known. The only data available, meager at best, are of a statistical nature.

In our experiments, measurements were taken on practically all surviving galls in 1920 (September, November), 1921 (April, December), 1922 (May, November), 1923 (April, August, December), 1924 (July, November), 1925 (May, June, October), the last-named year being the one in which the Experiment Station was abandoned. The series of inoculations here reported began in 1919. From that year onward the plants were kept under observation. An exact determination of the incubation period is impossible except by microscopical examination which entails destruction of, or at least serious interference with, the plants. Macroscopic examina-

tion of the initial stages, indicated by very slight swellings, is likely to be misleading. Furthermore, the great distance of the Experiment Station from headquarters in San Francisco eliminated the possibility of systematic and continued observation, so that the data here presented on the length of the incubation period are approximations. Since the Station was frequently visited during the period from spring to fall the plants were more closely watched during this time than during winter. The high mortality in the inoculated plants introduces a further weakness in the statistical material.

Peridermium harknessii.

Slit method. All inoculations were made in the greenhouse. The incubation period is here understood as the length of time elapsing between the date of inoculation and the first appearance of swellings indicating infection. The incubation periods expressed in months, as given in table 2, show

TABLE 2.—*Incubation period. Peridermium harknessii. Slit method.*

Number of swellings	Incubation period
6	3 months
21	4.6 "
2	8.5 "
2	10.3 "
3	13.6 "
32	14 "

great variation, although all plants were kept through the years under identical conditions. The type of host plants used varied very little. They were young potted plants ranging from about one-half to about two feet in height, with the exception of the series of very small seedlings of *Pinus coulteri* which has been referred to before.

The hosts used in this series were as follows: *Pinus sabiniana* to *P. jeffreyi, P. sabiniana* to *P. radiata, P. sabiniana* to *P. coulteri, P. contorta* to *P. contorta, P.* (*jeffreyi*) *contorta* to *P. contorta* and *P.* (*contorta*) *contorta* to *P. contorta.* The two last named are pedigreed, the name in the parentheses indicating the origin of the material used.

Spore shower. In this series part of the inoculations were made in the greenhouse, and part in the nursery. Since the conditions in the latter undoubtedly come closest to those prevailing in the open forest, one would expect the results to differ from those obtained under the artificial conditions of the winter-heated greenhouse. Table 3 gives the incubation period for the inoculations in the greenhouse and in the nursery. It may be well

to repeat that none of the hundreds of check plants in the nursery ever became infected. The following host plants were used in the transfers: *P. ponderosa-P. ponderosa, P. contorta-P. ponderosa, P. ponderosa-P. jeffreyi.* The aeciospore material employed in the greenhouse experiments came in part from Idaho, in part from British Columbia. All the aecial material used in the nursery tests came from Colfax, California, where *Peridermium harknessii* was first discovered. The only significant difference between the greenhouse and nursery sets consists in the uniformly much longer incubation period prevailing in the nursery. Obviously the artificial conditions in the greenhouse favor an earlier and more rapid development of the mycelium. All the nursery inoculations were performed on the same date (May 24, 1921). For the greenhouse experiments, with one exception (1 gall, 13.6 months' incubation), Idaho material was used. Inoculations made with the same aecial material show remarkably uniform results.

TABLE 3.—*Incubation periods in greenhouse and nursery. Peridermium harknessii.*

	Plants used	Swellings	Incubation period
Greenhouse	8	38	5 months
	5	7	11 "
	1	1	13.6 "
Nursery	6	23	23.5 "
	1	1	27 "

Peridermium cerebroides (?).

Slit method. Only a few inoculations by the slit method were made at the Experiment Station, all of which failed. It had been attempted to transfer the rust from *Pinus radiata* to *P. virginiana, P. thunbergii* and *P. densiflora.* Whether the failure was due to the lack of susceptibility of the hosts tested or to the very poor condition of the stock available remains uncertain. No swellings and galls developed.

Spore shower. A good series of galls was obtained by showering four plants in one pot (see Fig. 1). One of the plants remained sound. On the remaining three plants 13 galls developed which were observed and periodically measured through three years. The transfers were made in the greenhouse from *Pinus radiata* to *P. radiata,* the commonest host for *Peridermium cerebroides* (?). The inoculations, made on three plants, dated from March 27, 1920. On the first plant two swellings appeared after six months. The second pine showed four swellings after six months and a fifth swelling developed after more than twenty months from the date of inoculation. The third pine had five swellings six months after inoculation,

to which a sixth was added two months later. This plant died and is therefore not shown in Fig. 1.

GROWTH RATE OF SWELLINGS AND GALLS

The tabulation of growth figures is often difficult. Two neighboring galls on the same stem may grow together into one, or the swellings may extend into the branches or from the branches into the stem. New galls may appear from latent infections. Frequently the galls are so irregular in shape that their measurement rests upon uncertain ground. The majority of the swellings, however, develop according to type, forming galls of regular form and of the well-known shape.

Peridermium harknessii.

It would serve no purpose to give here the growth history of a great number of individual galls. In Fig. 2 the averaged measurements for three

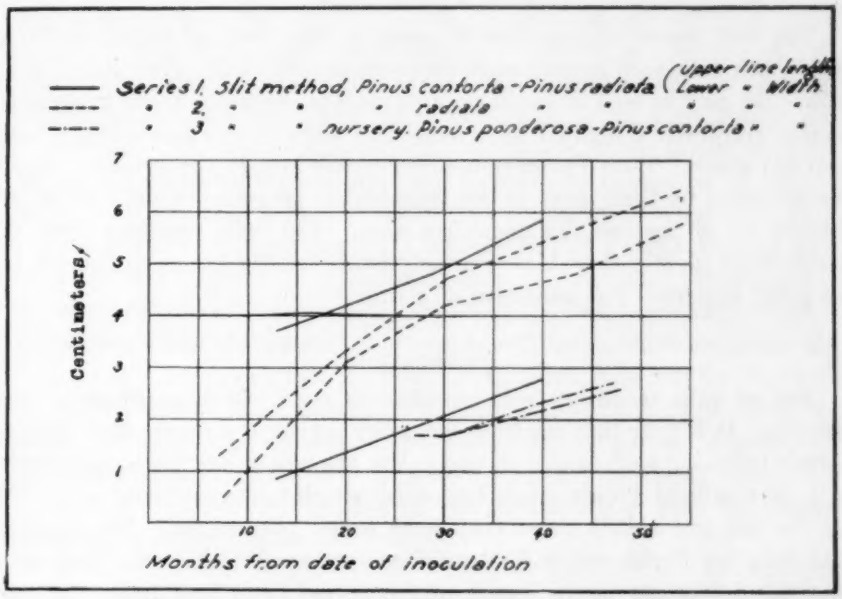

FIG. 2. Growth rate of galls, *Peridermium harknessii.*

series are given in centimeters over months elapsed since the date of inoculation. The upper lines represent height, the lower width. The figure shows plainly that the rate of growth for the galls grown in the nursery lags behind that of the greenhouse series. The highest of the averaged figures runs to 6.4×5.7 cm. after 54 months. Individual cases exceed these figures. One gall grew in 40 months to 9.0×2.7 cm., another in 61 months to 8.7×7.9

cm., and a third in 72 months to 7.4 × 6.8 cm. The most rapid growth was made by one gall which attained 18.4 × 1.4 cm. in 28 months.

Peridermium cerebroides (*?*).

No data on experiments by the slit method are available. As reported above only a few inoculations were made, all of which failed to produce galls.

Spore shower. In a series of experiments (*Pinus radiata-P. radiata*) conducted in the greenhouse (see Fig. 1) three out of four trees developed galls. The measurements of the galls were averaged and are presented below. The data are slightly disturbed through the late appearance of two new galls from latent infections. The inoculation took place on March 27, 1920; 7.6 months later the average dimensions were 1.4 × 6 cm. After 20.3 months they were 2.7 × 1.8 cm., and after 25.6 months 3.2 × 2 cm.

FORM OF THE GALLS

The first result of infection appears in the form of slight swellings which, as a rule, soon become more pronounced and finally grow into typical galls. As may be seen from the data on dimensions given above many galls remain atypically elongated for a long time, while others soon adopt a more rounded shape. Both *Peridermium harknessii* and *P. cerebroides* (*?*) are characterized by their more or less spherical to pear-shaped galls which are sharply set off against the unswollen stem. The galls resulting from the inoculations plainly show these characteristics, but the irregularities are far too great to permit the deduction of a rule.

SPORULATION

Not all galls resulting from inoculations reach the stage of aecial production. It is true that the high mortality among the plants used did not permit following each case to its end. But the writer has records of a series with four galls of *Peridermium harknessii* which never produced aecia during the six and a half years they were under observation. The sporulation data for *Peridermium harknessii* are summarized below. They show that the differences in the length of the period from inoculation to spore production do not seem to be induced by the ambient in which the infected plants were kept.

P. sabiniana-P. radiata.	Slit method,	greenhouse,	25, 25, 33	months
P. ponderosa-P. ponderosa.	Spore shower,	nursery,	25, 25, 25	"
P. ponderosa-P. jeffreyi.	"	"	"	24, 37	"
P. ponderosa-P. contorta.	"	"	"	24, 37	"

EFFECT ON THE INFECTED PLANT

Witches'-brooms. The formation of witches'-brooms is a characteristic of both *Peridermium harknessii* and of *P. cerebroides* (*?*). The stimulation of normally dormant axillary buds to vigorous growth is an almost constant corollary of infection and frequently becomes apparent at the time when the stem begins to swell perceptibly. The artificial infections differ in no way from those occurring in the wild state.

Stunting. Both *Peridermium cerebroides* (*?*) and *P. harknessii* have long been known to have a marked retarding effect on the height growth of the infected plants, which is most pronounced when the galls are located on the main stem. The artificially infected pines show the same stunting. Fig. 3 (*Peridermium cerebroides* (*?*), spore shower, *Pinus radiata-P. radiata*) illustrates strikingly the difference in height growth between infected and sound plants. At the beginning of this experiment all four plants were of the same height and in vigorous condition. Of the four plants in one pot, kept in the greenhouse, one escaped infection. Though crowded, it grew in three years and eight months to a height of 239 cm. Two of the infected plants in the same period only reached a length of 67 and 77 cm. respectively. The fourth plant was 61 cm. high when it died.

Killing. The statistics concerning death among the plants used for inoculations are so conflicting that they do not lend themselves to the formulation of a general rule. The writer has still in his possession a pine (*P. radiata-Peridermium cerebroides* (*?*)), the last of a series of 1914 which is now obviously declining. Another plant (*P. radiata-Peridermium cerebroides* (*?*)), inoculated in 1920 and bearing four large galls which completely surround the base of the tree, is still in vigorous condition though it is plainly stunted and its branching system has long lost its regularity.

Cultivation of pines in pots in the greenhouse is by itself likely to produce conditions unfavorable to the health of the plants. Undoubtedly a number of plants would have died even had they not been infected. The interpretation of mortality data of greenhouse plants calls for caution in assigning a definite cause for death.

A few clean-cut series may at least give indications. In April, 1920, eight small seedlings of *Pinus coulteri*, raised from seed in the greenhouse, were inoculated with aeciospores of *Peridermium harknessii* from *Pinus sabiniana*. Of these five developed pronounced galls and witches'-brooms. One died after one year, two after 16 months, the fourth after a year and a half and the fifth survived for over two years. The three uninfected plants were still in thrifty condition after five years. Since all the plants were kept under identical conditions, the cause of death may with safety be assigned to the infection. In another series already referred to (Figs. 1

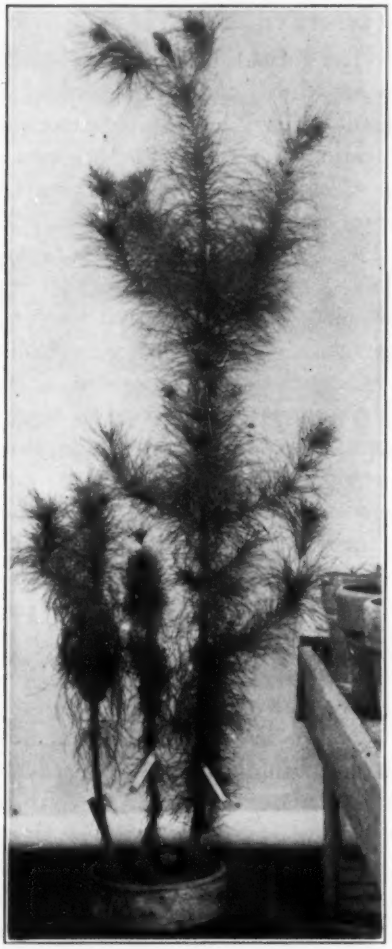

FIG. 3. Stunting of *Pinus radiata* infected with aeciospores of *Peridermium cerebroides* (?). One plant which escaped infection has grown to normal height.

and 3, *Peridermium cerebroides* (?), *Pinus radiata-P. radiata*) in which four plants in a pot were showered with aeciospores in the greenhouse, one escaped infection. This plant was alive and vigorously growing after six and a half years, while the three infected pines died within three and four years respectively after inoculation. The mortality is far lower in plants grown in the open.

MODE OF INFECTION

The writer had formerly expressed the surmise that infection took place through wounds, most probably through wounds caused by insects. Haack

and Hedgcock both followed the same thought. Klebahn's and the writer's successful experiments with showering unwounded pines with aeciospores seem to prove definitely that wounding is not necessary, while the writer's inoculations with the slit method in which aecial material is directly inserted in the tissues of the stem prove that wounds may serve as a means of entrance. The actual penetration of the germ tube into the host plant in the spore-shower experiments has not yet been observed. Klebahn (7, p. 203) asserts that the youngest shoots offer, without doubt, the principal means of entrance. The writer's results confirm, in the main, this view, but add to this the small spurs or "*Kurztriebe*" which in spore-shower infections seem to be subject in a higher degree to infection than the young shoots. In two series of inoculations (*Peridermium harknessii, P. ponderosa-P. ponderosa,* and *P. ponderosa-P. jeffreyi*) performed in May, the terminal buds of the host plants had sprouted out to a length of 2 to 4 inches. The young shoots were tender and the needle bundles had begun to stretch. One would assume that the young shoots were then in the most favorable condition for infection. Of the 16 plants showered with spores 7 produced 23 galls, all of which originated in spurs near the base of the plants. In no case had a young shoot become infected. In two other series (*Peridermium harknessii, P. ponderosa-P. contorta*) the showering with aeciospores had likewise taken place in May. Eighteen plants were used, of which 13 became infected and produced 72 galls. Of these 15 were located in the top on shoots growing out at the time of the inoculation, while 57 developed in the lower part of the plants, mostly from spurs attached to 2, 3, and even 4-year-old wood.

Klebahn (6, pp. 86–88) had proved that, in *Cronartium ribicola,* infection from sporidia could take place in the needles of *Pinus strobus.* Tubeuf (11) proved experimentally the infection through needles which he was able to follow through to the formation of aecia in the stem. In this country Clinton (2) later reported infection through the needles. It is interesting to note that Tubeuf (11, p. 282) found heavy infection taking place through spurs or *Kurztriebe* near the base of the host plant. He considers the primary leaves and incompletely developed buds, which are not thoroughly protected by bud scales, as particularly subject to infection. Klebahn, Tubeuf, and Clinton have called attention to the yellow spots appearing on the leaves and on the young tender stems as the first indications that the rust mycelium had actually become established.

In direct aecial transmissions the infection apparently does not take place through the needles. Though they were kept under careful observation, the needles of showered plants have never exhibited the characteristic yellow spots nor have such spots been found on the stem. The problem of the mode of entrance is still unsolved. Since Klebahn does not mention yellow spots for his inoculations with *Peridermium pini* it may safely be assumed that none were present.

PYCNIA

As has been reported before by the writer, pycnia have never been found for *Peridermium harknessii* or *P. cerebroides* (?) either in culture or in nature, with the single exception of one gall of *Peridermium harknessii* on *Pinus jeffreyi*. Harkness (5) had already stated that pycnia are unknown on *Pinus radiata*, *P. ponderosa*, and *P. sabiniana*, the three host plants then known for the mixed species *Peridermium harknessii* Moore. Eight years have elapsed since the writer's last publication on these two Peridermia. Not in a single case has he been able to find pycnia either in the experimentally produced galls or in wild material. This absence of pycnia in the two Pacific Coast forms, one of which is facultatively heteroecious while the other must now in all probability be regarded as autoecious, leaves no basis for speculation as to a possible meaning of the failure to produce pycnia. Artificially induced galls of *Peridermium pini*, which seems to be truly autoecious, produce pycnia, according to Klebahn. It seems likely that, for the two Pacific Coast forms, the production of pycnia is simply repressed but not completely suppressed and that internal pycnia may be found in relatively small numbers.

GERMINATION OF AECIOSPORES

The writer (8) has pointed out that the aeciospores, from either wild or cultivated galls, germinate readily and produce very long germ tubes which are occasionally branched but never show the slightest tendency to produce sterigmata or sporidia. Occasionally one spore sends forth two germ tubes. Dr. B. O. Dodge, in personal communications, has suggested to the writer that this branching may be interpreted in the sense of an abnormal or incomplete promycelium. Fig. 4 illustrates the phenomenon. The branches which frequently are as long as the main germ tube, generally originate near the base of the germ tube, and a septum is generally but not always formed near the angle of branching. In rare cases a second branch may be formed higher up, sometimes also accompanied by the formation of a septum, so that in some cases a two-celled, in others a three-celled structure results. It seems too early to ascribe to these the character of a promycelium. In no case, at any rate, has there been any suggestion of sporidia. The phenomenon is far from being general or even frequent. The fact that it is often totally absent suggests the possibility that it is in itself an abnormality induced by unfavorable moisture conditions in the moist chamber in which the slides with the germinating spores are kept. On the other hand, a septum often appears in the simple, unbranched germ tubes at a distance of two or three times the diameter of the spore. It is too early to speculate on the interpretation of this septum.

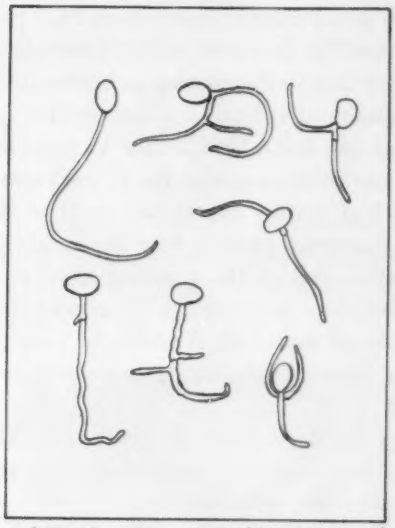

FIG. 4. Aeciospores of *Peridermium cerebroides* (*?*) germinating on a film of water. The germ tube not infrequently has one or two branches, often cut off from the main tube by a septum. Normally the germ tube is single and non-septate or one-septate.

In a former paper the writer has emphasized that the cytology of the mycelium from which the aecium arises, and of the spores formed by the latter, must be given due weight in deciding whether the spore with its germ tube is to be considered as a true aeciospore or as a modified teliospore.

At the writer's request Dr. R. H. Colley and later Dr. B. O. Dodge have had the courtesy to imbed, section, and stain material sent them. In both cases there is not the slightest doubt that the mycelium is uninucleate and that the spores are binucleate. It is difficult to imagine, in the face of these facts, that we are dealing with a teliospore which has lost all the characteristics of a teliospore.

SUMMARY

The gall-forming Peridermium common on *Pinus radiata* and other coastal pine species of California is now believed to be not *Peridermium cerebrum* Peck but a form of unknown relationship, tentatively called *P. cerebroides* (*?*). The gall-forming Peridermium common on *Pinus contorta, P. sabiniana, P. ponderosa,* and other pines of the interior mountain ranges of California is maintained as being the aecial form of *Cronartium harknessii,* nov. comb. This *Peridermium harknessii* has again experimentally been proved to go to Castilleia as the alternate host. The same aeciospore material is, however, equally able to infect pines directly. The author's previous results are confirmed by a large number of experiments, 70 per cent of which were successful for *P. harknessii* when the slit

method is used. With spore-shower inoculation 78.5 per cent were successful in the greenhouse and 44 per cent in the nursery. *Pinus coulteri* has been proved to be susceptible to *Peridermium harknessii* infection. *P. cerebroides* (?) also is capable of infecting pines by the spore-shower method. The incubation period for both Peridermia varies considerably. In the experiments reported the periods varied for *P. harknessii* between 3 and 14 months by the slit method and by the shower method from 5 to 27 months. Data are given on the average growth rate for artificially produced galls, on the time of sporulation and on the stunting effect of the infections. Infection apparently takes place not through the needles but through the stem, probably through buds of spurs or *Kurztriebe* poorly protected by bud scales. As in former experiments no pycnia have been found either in artificial or wild galls.

The germination of the aeciospores is discussed. The branching of the germ tube which is not infrequent suggests at least an approach to a promyceliar type. No sporidia have been observed.

OFFICE OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
SAN FRANCISCO, CALIFORNIA.

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POKKAH-BONG AND TWISTED TOP DISEASES OF SUGAR CANE IN CUBA

C. N. Priode[1]

INTRODUCTION

During the last few years a disease of sugar cane, which appears to be identical with the Pokkah-bong disease in Java, has been under observation in Cuba. So far as is known it has not, heretofore, been reported from the Island, although it was observed and recognized in November, 1924, at

Fig. 1. Cristalina plant affected with Pokkah-bong. Note the splitting and breaking over of the leaves. Photographed Nov., 1924.

Central Santo Tomas, Provincia de Camagüey, by Dr. J. A. Faris, Chief Pathologist of this Station.

Doctor Faris observed a number of Cristalina plants with chlorotic leaf bases and damaged tops. A closer examination revealed the presence of red

[1] Assistant Pathologist, Cuba Sugar Club Experiment Station.

markings on the chlorotic parts. Infection on some of the plants had advanced to the rot stage, causing the death of the whole plant tops. Photographs were made of some of the affected plants, one of which is shown in figure 1. Specimens of the affected leaves were collected and preserved in the herbarium at the Baraguá Laboratory.

Although the disease was not observed until 1924, it probably has been present in the Island for many years. The varieties generally grown in Cuba previous to that time were not particularly susceptible to the attacks of the disease, hence it is quite probable that no serious outbreak ever occurred.

However, with the importation of susceptible new varieties during the last few years, the disease has made itself manifest in several different localities over the Island and, during seasons favorable for its development, it may prove to be of considerable economic importance.

TWISTED TOP

While investigations were being made into the different stages of the above disease, a few plants were observed with peculiarly twisted tops which appeared to be somewhat different from the symptoms of Pokkah-bong. At first, this affection was thought to be a stage of the Pokkah-bong disease but careful examinations indicated it to be a separate and distinct disease, probably identical with the Pokkah-bong or twisted-top disease in Hawaii. The leaves of the affected plants were twisted and tangled but had the color and texture of normal leaves. The twisting of the leaves was the only visible symptom and appeared to be caused by the older leaves clinging together and forcing the younger leaves to grow through them.

This disease probably has existed in the Island since sugar cane was first introduced. Its occurrence on the varieties generally grown in Cuba is so slight that it is of little consequence at present. However, should sensitive varieties be imported, it might, during seasons favorable for its development, prove to be more important. From the following description it will be seen that these two maladies have been reported from other countries as one disease. In a previous report by the writer (6), however, attention was called to the fact that they were separate and distinct from each other.

In 1898 Wakker and Went (7)[2] described and illustrated a disease of sugar cane in Java which they called Pokkah-bong because of the twisted and damaged condition of the affected tops. (Pokkah-bong is a Javanese term which means damaged top shoots.) This was the first report on the disease and very little was known about it at that time. The symptoms as described by them are as follows:

[2] The numbers in parentheses refer to literature cited.

"During the beginning of the rainy season when the cane is young, plants are often found which have a different appearance to the rest of the cane plants in the field. The young partially unfurled leaves show yellow to almost white spots on or near their bases. Rows of holes or cracks usually develop on the white spots. The tips of the leaves are usually green and healthy.

"Sometimes not more than one leaf shows these peculiarities but other cases may be seen where several leaves coming next to each other are infected. Keeping track of these shoots one may observe that the affected leaves do not develop in the usual manner but either break off or split through the affected parts. Dark red to black stripes sometimes develop on the affected leaves. Usually the shoots keep on growing without further damage. Closer examinations of the stalks show them to be somewhat thinner at the points of attachments of the affected leaves. As the disease becomes more severe the internodes under the diseased leaves become funnel shaped, *i.e.*, they are thicker at the tops than at the bases. These internodes are usually very much shorter than the normal ones. Sometimes roots start growing at these short internodes giving the plant somewhat the appearance of a Sereh diseased stick and doubtless this disease has been mistaken for Sereh many times. It is not impossible that both appear at the same time but when it is a case of Pokkah-bong only, the red Sereh stripes do not appear in the joints." (Translation from the Dutch.)

Wakker isolated several species of bacteria but was unable to reproduce the disease from his inoculations with them. The negative results from these inoculations, together with his observations that the disease developed most severely during the warm damp weather which accompanies the change from the dry to the wet season, caused him to conclude that the disease was not of parasitic origin but was the result of the slower-growing outer parts of the leaf funnel, squeezing the rapidly-growing inner parts. This belief was firmly established in Java until the summer of 1927 when Bolle made investigations into the different stages of the disease (1). Observations on the occurrence and spread of infection in the field led to the belief that the disease was of an infectious nature. Bolle's description of the symptoms of natural infection is very similar to that of Wakker and Went, as is seen from the following translation of a recent report (2):

"The first symptoms of Pokkah-bong appear as chlorotic spots on the young leaves, especially near the bases. The appearance of the chlorotic spots is usually followed closely by a malformation of the affected parts. In severe cases the leaves do not unfold normally but are more or less stuck together and are folded, wrinkled, and curled. Red markings frequently appear on the chlorotic parts and these sometimes develop into lens- to rhomb-shaped holes.

"These holes are not easily mistaken for borer holes, as they can easily be distinguished from them partly by the form and partly by the location. The holes caused by borers are circular or round and lead directly through the leaf sheath into the stalk while those caused by Pokkah-bong are as above mentioned: lens- to rhomb-shaped and spread unsystematically over the chlorotic parts, or form rows in the longitudinal direction. The holes formed in the latter way sometimes run together forming long ladder-shaped depressions. The red markings, whether in the shape of holes, spots, or streaks, may continue down into the leaf sheath.

"From the youngest leaves the attack sometimes continues down into the stalk by way of the buds and growing points and dark red streaks may be found extending through several joints. These streaks appear in the joints as fine lines but between the joints they sometimes widen to form long depressions with cross partitions which give them a ladder-shaped appearance.

"The attacks sometimes cause a stunting of the growth by which the internal affected parts can often be recognized, due to the short lengths of the internodes. When the ladder-shaped markings appear at one side close to or at the surface of the internodes the growth is checked only on one side which causes a curved internode.

"When a leaf sheath is strongly attacked a rotting develops on the inside which may pass to the stalk. Here the rot may remain for some time limited to a dry rot of the growing points and the very young internodes. This rotting of the leaf sheath and stalk has often been confused with borer attacks and, where the stalks are badly stunted and the symptoms remain inside the leaf sheath, it is sometimes very difficult to distinguish between the two at first sight. When the stalk is cut lengthwise, however, the difference is very readily seen.

"The above-mentioned symptoms are not always equally developed. Sometimes the deformity of the chlorotic parts is most noticeable, in other cases the red markings are most conspicuous. Also, the different varieties show a great difference both as to severity of attacks and symptoms concerned."

In April, 1927, Edgerton and Tims (3) reported a disease of sugar cane from Louisiana which they believed to be the same as the Java Pokkah-bong. Their description of the symptoms of the disease is short but agrees very well with that of Wakker and Went for the disease in Java. They state as follows:

"A disease identical with or similar to the one known in Java as Pokkah-bong, a Javanese term meaning twisted top, has in recent years made its appearance in Louisiana. The identity of the Louisiana disease with the one in Java was confirmed by H. Atherton Lee, of the Hawaiian Sugar

Planters Experiment Station, while making a trip through Louisiana in the summer of 1926. While the disease was very common during the summers of 1925 and 1926, as far as is known, this is the first published report on it.

"Pokkah-bong first shows in the new leaves as they come out of the terminal bud. The leaves are light colored and are much twisted, frequently to such an extent that they are practically tied together. Sometimes these young leaves rot and, on this account, the disease has frequently been called top rot in Louisiana. Usually, however, the leaves grow partially out of this twisted condition. New leaves that develop later may show little signs of the disease.

"The internodes which form in association with the twisted leaves very frequently remain short. The disease was first observed on the newly introduced Java seedlings, but later appeared on the Purple, D 74, and other Louisiana varieties."

In January, 1928, Lee (5) reported a Pokkah-bong or twisted-top disease from Hawaii, which he states is a non-infectious disease caused by the friction of the leaf hairs which prevents the leaves from unfolding normally. He writes as follows:

"One notices that some of the tall cane stalks appearing above the usual height of the cane fields do not have their leaves expanded normally but a mass of closely wrapped, twisted ribbons of leaves. Examining more closely one will observe that many of the leaves are split and the growing mass of the cane tops is wrapped around by such split leaves. The outermost leaves of the bunched cane tops are of the usual green color of normal leaves but the younger, inner leaves are of a greenish-yellow color. Moreover these younger, inner leaves are not of the usual texture of normal leaves but are buckled and corrugated and have somewhat the character of crumpled cabbage leaves. These inner leaves are very rigid and seem to be under considerable mechanical tension. Less advanced stages of the disease are sometimes seen in which the whole tops of the plants are not tied up but the tips of the leaves as they emerge from the growing point do not separate from each other. Such cases with the lower parts of the leaf blades distended but tied together at their tips have an arched appearance."

It seems evident from the above reports that there are two separate and distinct diseases described under the name Pokkah-bong. The Pokkah-bong diseases in Java, reported first by Wakker and Went and later by Bolle, probably are identical. The symptoms described in each report are very similar and the colored plates given by these workers portray stages of the disease which strongly suggest a parasitic origin. It is also quite probable that the trouble reported from Louisiana is of parasitic nature and identical with the disease in Java.

The Pokkah-bong or twisted-top disease reported by Lee from Hawaii, however, appears to be a distinctly separate malady. The symptoms described do not suggest a parasitic origin but, as shown by Lee, indicate the

FIG. 2. Pokkah-bong infection on B H 10/12 plant, showing ragged appearance of leaf bases caused by the falling out of the rotted areas after they became dry.

twisting of the tops to be caused by a simple mechanical friction of the leaves which prevents their normal separation. This also is true of the twisted-top disease in Cuba. The symptoms of Pokkah-bong in Cuba, however, are very similar to those of the Java disease, as the following description will show.

DESCRIPTION OF THE POKKAH-BONG DISEASE IN CUBA

The first symptoms of Pokkah-bong usually appear as chlorotic spots on the bases of the young leaves. These spots become apparent as the leaves emerge from the leaf spindle. Infection appears to take place in the young leaves of the spindle, probably while it is full of water.

In cases of light infection the chlorotic spots are the only symptoms. Usually, however, red markings develop on the chlorotic parts before the leaves are fully unrolled and, when the attack is very severe, may result in a wet rot. After the leaves separate from the leaf roll the red markings may dry and fall out, giving the leaf the ragged appearance seen in figure 2.

FIG. 3. Plant affected with Pokkah-bong, showing how the old chlorotic leaves become corrugated and drawn, forcing the younger leaves to buckle and split their way through to the outside.

Sometimes such leaves break over and fall off. Where the infection reaches down into the very young tissues of the leaf-roll, a rot sometimes develops which may destroy the whole leaf spindle down to the growing point. Usually, however, the younger, inner leaves grow through the affected parts without being seriously damaged.

Some cases have been observed where the chlorotic parts of the outside leaves become wrinkled and drawn and do not unfold normally but cling tightly together, forcing the rapidly growing, younger leaves to buckle and split their way through to the outside, as in figure 3.

In very severe cases the leaves become so wrinkled, corrugated, and drawn that they cannot unfold but split and twist around each other, forming a closely wrapped mass of the whole plant tops. Figure 4.

On susceptible varieties infection is not limited to the leaves but may pass from the leaves and leaf sheaths down into the stalks. When the stalks are split open, red streaks can be seen extending from the points of attach-

FIG. 4. A severe case of Pokkah-bong on a POJ 2714 plant. The corrugated and drawn leaves have tied the top into a closely wrapped mass. Note the ragged appearance of the older leaves where the red markings have fallen out.

FIG. 5. Section of a POJ 2878 stalk infected with Pokkah-bong, showing the surface cavities. When such stalks are split open red lines or streaks extending down into the stalk can usually be seen.

ment of the affected leaves far up into the stalks. These red lines sometimes widen between the joints to form long sunken cavities, which usually cannot be detected until the stalk is split open. Sometimes, however, they are evidenced by the short lengths and bulgings of the affected internodes.

In some cases the infection does not penerate very far into the stalk but is confined to the surface tissues. Here, the cavities may be observed extending from the base of an infected sheath, up the outside of the stalk. A section of an infected stalk showing the surface cavities is shown in figure 5.

Sometimes the cavities develop on the part of the stalk enclosed by the leaf sheath and may pass unobserved until the sheath is removed. Their presence can usually be detected, however, by the curved internodes which result from the uneven growth on the sides of the infected internodes.

Any or all of the above symptoms may occur on the same plant. The chlorotic spots on the leaves, however, are usually the first and most conspicuous symptoms by which the disease may be recognized. These symptoms of Pokkah-bong are very clearly illustrated by the colored plate (Plate I) in this paper. The disease usually makes its appearance shortly after the beginning of the spring rains and continues its attacks throughout the rainy season. During dry periods it does not appear to be of much consequence.

FIG. 6. A plant affected with twisted top, showing how the young leaves are held together by the split older leaves.

DESCRIPTION OF TWISTED TOP IN CUBA

Twisted top, as it occurs in Cuba, affects only the plant tops. The older leaves appear to be held together by some mechanical means and do not separate normally. The growth of the younger leaves from below usually causes the older leaves to split and tangle. These in turn have a tendency to wrap around the young leaves and the whole plant top may grow into a tangled mass of split leaves. Sometimes the younger leaves of the leaf-roll grow through the split, older leaves but are so closely wrapped by them that they do not unfold but grow into a slender spike-like mass of wrapped leaves, as in figure 6.

Other cases may be seen where the spindle of the plant is not wrapped around by the older leaves but the tips of the leaves cling together and do not separate. As the growth continues from below, the leaves form bows or arches. In some cases these arches begin to form when the plant is young and, as each new leaf emerges from the leaf-roll, its tip becomes enmeshed

FIG. 7. Twisted top, showing how the tips of the leaves become entangled, forming arches as the growth continues.

with the tips of the older leaves and an additional arch is formed. This process may continue until all the leaves on the plant will be formed into a series of arches, one over the other, on one side of the plant. Figure 7.

No parasitic symptoms were observed on any of the plants affected with twisted top. The color and texture of the affected leaves were the same as for normal leaves.

Unlike Pokkah-bong, the disease occurs mostly during the dry seasons; only a few scattered cases may be seen during the rainy periods.

The dissimilarity of the above symptoms of the two diseases indicates Pokkah-bong to be a disease separate and distinct from twisted top. The results obtained from the following experiments, however, will make this fact more evident and more clearly understood.

EXPERIMENTS WITH THE POKKAH-BONG DISEASE IN JAVA

Bolle's observations on the occurrence and spread of infection in the field led her to believe that the Pokkah-bong disease in Java was of parasitic nature. Accordingly, she made several isolations from the discolored vascular bundles in stalks affected with Pokkah-bong. In nearly all her cultures she obtained the same Fusarium later identified by Wollenweber as *Fusarium moniliforme* Sheldon. Other species of fungi and several bacteria also were isolated but none of these proved infectious when inoculated onto healthy plants. Inoculations made from pure cultures of the Fusarium fungus, however, produced typical symptoms identical to those she observed on naturally infected plants in the field.

On one set of plants the inoculum—a suspension of spores in distilled water—was dropped into the leaf funnel from a pipette. Only leaf symptoms developed from these inoculations. Chlorotic spots and red markings soon appeared on the bases of the young leaves but no stalk symptoms were observed. On the other set of plants the inoculum was injected into the heart of the leaf-roll, just above the vegetative point, by means of an injection syringe. By this method the spores were put in direct contact with the young, tender tissues of the leaf funnel and, from these inoculations, both leaf and stalk symptoms developed. The chlorotic spots and red markings soon made their appearance on the bases of the leaves and from these the red lines could be seen extending down into the stalk. In some cases the red lines widened between the joints to form the ladder-shaped figures or cavities characteristic of the advanced stages of stalk infection. On some of the plants, infection resulted in severe cases of top rot, in which the whole of the leaf rolled and the growing point died.

About two and one-half weeks after the inoculations were made the same Fusarium was reisolated from infected tissues far above the point of injection. Inoculations made from the reisolated cultures produced typical Pok-

kah-bong symptoms. These inoculation experiments were repeated several times on different varieties of cane with similar results.

EXPERIMENTS WITH THE POKKAH-BONG OR TWISTED-TOP DISEASE IN HAWAII

In his experiments with the Pokkah-bong disease, in Hawaii, Lee mentions no attempt to isolate any causal organism. His observations on the occurrence of the disease in the field, together with a remark by Mr. Norman King, agriculturist for the Pioneer Mill Company, that there seemed to be some cause of friction as the leaves were emerging from the central cylinders of the cane tops and that this friction caused a buckling of the rapidly growing young leaves, led him to believe that the trouble could be produced artificially by increasing the friction on the young leaves. This belief was strengthened by a previous study of leaf structures by Lee in which he observed the presence of short, rigid hairs or bristles on the leaves of H 109 plants. (H 109 was the variety most severely affected by the disease.)

To test this theory Lee selected several plants of the H 109 variety and bound the leaf spindles with ordinary black friction tape. The purpose of this was to increase the friction on the rapidly growing young leaves. A fine reproduction of twisted tops developed from this increased friction on nearly all the plants so treated. The leaves became corrugated and enmeshed at their tips, presenting an arched appearance as they emerged, and they could not separate. The next emerging leaves were obstructed by the arched entanglements of the older leaves and were forced to grow through them, causing them to split and twist. The split, older leaves then had a tendency to tie up and increase the friction on the younger leaves so that the whole top growth usually resulted in a closely wrapped mass of split leaves.

In another experiment he produced typical cases of twisted tops by abruptly bending the leaf spindles about middle ways of their heights. These experiments were repeated with like results in each case. Lee makes no mention of the parasitic symptoms characteristic of the Pokkah-bong disease in Java. The twisting and tangling of the leaves appeared to result from the leaf hairs clinging together and preventing the normal separation of the leaves.

In concluding his report Lee makes two statements, as follows:

(1) "It seems evident that twisted top as it occurs in Hawaii and probably in Louisiana, is a mechanical difficulty and non-infectious."

(2) "The writer at first inclined toward the feeling that the Java and Philipppine form of the disease was infectious and distinct from this form common in Hawaii. With the development of this work, however, one begins to feel that the form in Java and the Philippines might simply be a more severe form of this mechanical injury. One would at least feel doubtful in considering any form of this disease as infectious."

It is evident from the results of these experiments by Lee that the twisted-top disease in Hawaii is a non-infectious disease caused by the mechanical friction of the leaf hairs, as described in his report. It is also evident from his conclusions, that Lee considers the disease to be the same as, or a different stage of, the Pokkah-bong disease in Java. These widely different experimental results obtained by Bolle in Java and Lee in Hawaii seem to indicate that they were dealing with two separate and distinct maladies under the same name. To determine whether this was the case the following experiments were undertaken:

EXPERIMENTS WITH THE POKKAH-BONG DISEASE IN CUBA

The experiments with the disease in Cuba were performed at this station and were carried out as follows:

Experiment 1.—One row of the seedling variety of Baraguá 2 was selected for the experiment. All the plants in the row were closely examined but no evidence of the disease was observed. This variety does not easily become infected with Pokkah-bong under natural field conditions. Six plants of one stool were inoculated with Fusarium spores from a pure culture isolated from the red lines deep down in a POJ 2878 stalk affected with Pokkah-bong. A suspension of the spores in distilled water was injected into the center of the leaf funnels, just above the growing points, by means of an injection syringe.

Six other plants of the same stool were inoculated in the same way, except that distilled water alone was used for the inoculum. These latter plants, together with the uninoculated plants in the row, served as checks. About two weeks after date of inoculation the plants were examined and five of the six stalks showed typical symptoms of Pokkah-bong infection. These symptoms appeared strikingly similar to those described by Bolle from inoculations with the Fusarium spores in Java. One of these plants is shown in figure 8.

The chlorotic spots on the leaves were the first symptoms to appear. These were followed very closely by the red markings. Infection in two of the plants had advanced to the top-rot stage and the leaf-rolls of these plants were completely dead. No stalk symptoms developed on any of these plants.

None of the checks inoculated with distilled water alone showed any symptoms of infection and none of the untreated plants in the row were infected, although plants of other varieties in adjoining rows were severely infected.

Experiment 2.—The above experiments were repeated on other varieties of cane. The same Fusarium fungus was reisolated from the plants artificially infected in the first experiment. Spores from these new isolations were injected into a few plants of each of the following varieties: FC 306;

FIG. 8. Pokkah-bong produced from inoculations with Fusarium spores. Note the markings on the chlorotic parts.

BH 10/12; D 109; SC 12/4; PR 525; and Cristalina. Checks were kept for each of the varieties except PR 525. All the varieties inoculated showed symptoms of Pokkah-bong infection within ten days from date of inoculation, though not all the plants of each variety became infected.

The red markings and chlorotic spots were very pronounced on all the plants infected. One plant of FC 306, one of BH 10/12, one of SC 12/4 and two of PR 525 showed severe cases of top rot. The leaf rolls of these plants were completely dead. None of the check plants showed any infection. The plants in this experiment were in pots and were kept in cages inside the plant house where the possibility of infection from wind-borne spores was greatly reduced.

Experiment 3.—Observations on the natural occurrence of the infectious type of Pokkah-bong in the field showed that none of the varieties used in the above experiments is particularly susceptible to the attacks of the disease. Very few cases of natural infection were observed on these varieties. The variety POJ 2878 was reported from Java to be very susceptible to the disease. But no plants of this variety were available at the time the above experiments were made. However, fourteen plants were secured later and seven were inoculated with Fusarium spores on August 8. The spores were injected into the leaf-rolls with the injection syringe, as in the above experiments. The other seven plants were used as checks. The plants were examined on August 20. All of those inoculated with the spores were severely infected with Pokkah-bong. The bases of the leaves showed the chlorotic spots and red markings very plainly. Infection on four of the plants had advanced to the top-rot stage and on these the spindle rolls were dead down to the growing points. Figure 9. None of the check plants were infected.

The results from these inoculation experiments leave little doubt that the Pokkah-bong disease in Cuba is infectious. However, to remove all possible chances of outside infection, inoculations were made with the Fusarium spores under absolutely aseptic conditions.

The same method was employed as that devised by Faris for his inoculation experiments with the brown-stripe and eye-spot diseases of sugar cane (4). The experiment was performed as follows:

Experiment 4.—Fifteen glass cylinders, 50 centimeters high by 10 centimeters in diameter, were filled with sand to a height of from 15 to 20 centimeters. (The sand had previously been washed for a period of six hours to remove the excess salts.) It was then watered with a chemical solution suitable for cane growing. The cylinders were plugged with cotton, wrapped with ordinary wrapping paper, and sterilized in a large autoclave under 25 lbs. pressure for five hours. Smaller cylinders also were filled with sand, wrapped, and sterilized with the larger cylinders.

Single-bud seed pieces of the variety POJ 2727 were soaked in a 1/1000 solution of mercuric bichloride for 15 minutes. They were then transferred directly to the large cylinders and covered with the sand from the small tubes. The cotton plugs were replaced and the tops of the jars rewrapped

FIG. 9. Pokkah-bong produced by injecting the Fusarium spores into the heart of the leaf-roll. The central stem is completely dead.

with paper. The whole procedure of planting the cane in the jars was done in an aseptic inoculating chamber.

When the cane had germinated and the plants had reached a height of from eight to ten inches, they were inoculated with spores from a pure culture of the Fusarium fungus, as follows: The jars were placed in the aseptic inoculating chamber and the cotton plugs removed just enough to allow the insertion of an atomizer tube. Twelve of the plants were then thoroughly sprayed with a suspension of the spores in distilled water, the cotton plugs replaced and the tops again wrapped with paper. The three uninoculated plants served as checks.

About 15 days after date of inoculation there were no definite symptoms of Pokkah-bong on these plants. There were faint chlorotic spots on the leaves of some of the plants but these could not be taken as definite Pokkah-bong symptoms.

The jars were again brought into the inoculating chamber and the plants were inoculated a second time. This time the spores were injected into the young tissues of the leaf-rolls by means of the injection syringe.

Twelve days after inoculations were made, three of the plants showed symptoms of infection. After fifteen days, two of these plants had developed severe cases of top rot and the spindle rolls were completely dead. The other plants together with the checks remained free of the disease.

These results prove definitely that the Pokkah-bong disease in Cuba is infectious and caused by a species of Fusarium. The symptoms produced from the above inoculations were identical to those described by Bolle from her inoculations with the Fusarium spores in Java and leave little doubt that the two diseases are identical.

EXPERIMENTS WITH TWISTED-TOP DISEASE IN CUBA

In these experiments no attempt was made to isolate any causal organism. No parasitic symptoms were observed on any of the plants in the field, so the disease was not believed to be of parasitic origin but closely related to, if not identical with, the twisted-top disease in Hawaii. The experiments were performed as follows:

Experiment 1.—Six plants in each of two stools of the variety Baraguá 2 were selected for the experimental work. The spindles of the plants in stool No. 1 were bound near the base with ordinary black friction tape. The spindles of the plants in stool No. 2 were bent abruptly about middle ways of their heights. About three weeks later all of the plants with the wrapped spindles, and all with the bent spindles, showed varying degrees of twisting. On some of the plants the tops were intricately tied up. The older leaves had a tendency to cling together and were split by the growth of the younger leaves from below. The younger leaves were then wrapped around by the split, older leaves and the whole top growth usually resulted in a mass of split and tangled leaves. Figure 10.

In most of the cases where the spindles were wrapped with the friction tape the leaves did not split or tangle but the tips were held together, forming arches similar to those described by Lee (5). As the new leaves emerged from the leaf spindle their tips became enmeshed with the tips of the older leaves, and additional arches were formed, all on one side of the plant. In some cases the tips of the leaves died back to the point where the tape was wrapped around them. Figure 11.

There were no parasitic symptoms connected with these, however, and the dying back appeared to be caused by the tight wrapping of the tape. No chlorosis nor red markings were observed on any of these plants nor were there any indications of top rot.

FIG. 10. Twisted top produced by the abrupt bending of the leaf spindle when the plant was young. Note the ribbon-like strips of the split, older leaves.

In another experiment ten plants of the seedling variety Baraguá 10 were selected. The leaf spindles of all ten plants were bent abruptly about middle ways of their heights. About two weeks later all of these plants had developed typical cases of twisted tops. The bending of the leaves appeared to hold the leaves together in such a way that the hairs enmeshed and would not separate. The older leaves, becoming entangled, obstructed the growth of the younger leaves. These in turn either split the older leaves and grew through them or became entangled with them and formed the arches described above.

The results of these experiments confirm the work of Lee and prove that the twisted-top disease in Cuba is non-infectious and identical with the twisted-top disease in Hawaii.

POKKAH-BONG AND TWISTED TOP SEPARATE AND DISTINCT DISEASES

The results of the above experiments also prove Pokkah-bong and twisted top to be separate and distinct diseases. Each has its own distinctive symp-

FIG. 11. Twisted top produced by wrapping the young leaf spindle with friction tape. The tape can be seen at the bend of the first leaf. The tips are dead back to this point.

toms which readily distinguish it from the other. The following comparison of these symptoms will make this fact more evident and more clearly understood:

Symptoms of Pokkah-bong

1. Affected tops may become twisted, corrugated, and drawn.

2. Bases of affected leaves usually chlorotic, with red markings.

Symptoms of twisted top

1. Top leaves split, twist, and tangle, but the texture remains the same.

2. Color of affected leaves same as for normal leaves.

Symptoms of Pokkah-bong	*Symptoms of twisted top*
3. Leaf sheaths may show red markings, with cavities or holes.	3. No symptoms observed on leaf sheaths.
4. Top rot sometimes develops which destroys the whole leaf spindle.	4. No indications of top rot.
5. Red streaks and cavities may develop on and in the stalks of affected plants.	5. No stalk symptoms observed.
6. Attacks most severe during rainy periods.	6. Affection most severe during extremely dry periods.
7. Caused by species of Fusarium.	7. Caused by simple mechanical friction of the leaves.

The above comparison shows that the only distinguishing symptom of the twisted-top disease is the twisting and tangling of the affected leaves. No sheath or stalk symptoms were observed. The symptoms of Pokkah-bong, however, are numerous. The leaves, leaf sheaths, and stalks may all become infected and show the parasitic symptoms.

The only symptom common to both diseases is the twisting or damaging of the plant tops. This one symptom, due to the fact that the diseases have been but vaguely understood, led to the belief that they were the same or different stages of the same disease. Where the two maladies occur on adjoining plants, as was the case in some of the experiments performed by the writer, their differences can readily be seen. Each has its own distinctive type of twisting and one can readily be distinguished from the other.

DISTINCT NAMES SUGGESTED FOR THE DISEASES

The name Pokkah-bong, as stated in another part of this paper, was given to the disease in Java by Wakker and Went, in 1898. Since that time numerous reports of Pokkah-bong diseases have been published from many parts of the world. These, for the most part, have been but vaguely understood and we have no assurance that they were the same in each case as the Pokkah-bong disease described and illustrated by Wakker and Went. It is now known that the twisted-top disease in Cuba, which appears to be identical with the Pokkah-bong disease reported from Hawaii, is a trouble separate and distinct from the infectious Pokkah-bong and it is quite probable that some of the other diseases reported as Pokkah-bong are of like nature.

Where the diseases occur in mild form in a field, an observer, not knowing the existence of the two diseases and not being familiar with the characteristic symptoms of each, would perhaps mistake them to be the same or different stages of the same disease. Now that they have been proved to be two separate and distinct maladies it is necessary that each be given a specific name, so that they can be recognized and distinguished from each other.

Since the term Pokkah-bong was first given to the malady in Java, it is the opinion of the writer that this name should be given exclusively to that disease and another name be selected for the non-infectious disease.

The disease in Hawaii has been called "Topknot," "Twisted Top" and various other names descriptive of the twisted and tangled condition of the affected tops, and since this twisting is the most conspicuous symptom of the disease the term twisted top naturally suggests itself as the most appropriate name for the disease.

The adoption of these names will probably clear away the confusion which has heretofore surrounded these two maladies and will serve to distinguish one from the other when they are observed in the field.

VARIETAL RESPONSES TO THE DISEASES.

Pokkah-bong

The varieties of cane commercially grown in Cuba at the present time appear to differ greatly in their susceptibilities to the attacks of Pokkah-bong. Most all of them are susceptible to a certain extent, but on some varieties the symptoms of the disease are barely noticeable.

The Cristalina and D 109 varieties contract the disease very readily but infection is usually confined to the leaves. The chlorotic spots and red markings may be very pronounced on the leaves and a few cases of top rot may develop during favorable weather but the loss from the disease on these varieties is very small. No indications of stalk symptoms have been observed on either of these varieties, though they might develop under more favorable conditions.

The varieties SC 12/4, FC 306, BH 10/12, Yellow Caledonia and some of the Porto Rican seedling canes also are somewhat susceptible but the attacks on these varieties are usually very light, with little resulting damage.

Most of the newly introduced Java canes are more or less susceptible to the disease. Some of these show only faint, chlorotic symptoms on the bases of the leaves; while on others, the attacks are more severe. The POJ 2878 variety appears to be most severely infected of any of the varieties so far observed in Cuba. In a recent report by Bolle (2), POJ 2722 and POJ 2878 are given as the most susceptible varieties in Java.

On the experiment plots of this station, where the overhead method of irrigation is employed, about 60 per cent of the plants of the POJ 2878 variety became infected. The regular overhead sprinkling of the plants kept the tops moist and made conditions especially favorable for the development of the disease. Some of these plants showed only the chlorotic leaf symptoms, while on others the infection had advanced to the top-rot stage,

killing the leaf-rolls and growing points. The loss on these canes from top rot, however, was very small. On a few plants of this variety infection had extended down into the stalks, forming the cavities described and illustrated in another part of this paper. Under ordinary field conditions the percentage of infection on this variety probably would be very much less than was observed on the irrigated plots.

The POJ varieties 2727, 2714, and 2883 are also susceptible to the disease but to a much less degree than POJ 2878. Infection on POJ 2725 was observed to be very slight.

Twisted top

Twisted top does not seem to be limited to any particular variety but develops on most any plant where the friction of the leaves is increased. The few cases of natural occurrence in the field, observed during the past summer, were widely scattered and, in nearly every case, resulted from some injury to the plant tops.

SUMMARY

The symptoms of Pokkah-bong and twisted-top diseases are described and illustrated. Experiments were performed which proved them to be separate and distinct diseases.

Pokkah-bong is shown to be an infectious disease, probably identical with the Pokkah-bong disease in Java. Experiments proved it to be caused by a species of Fusarium, very similar to *Fusarium moniliforme* Sheldon described as the organism causing the Pokkah-bong disease in Java.

Twisted top is a non-infectious disease probably identical with the Pokkah-bong disease reported from Hawaii. It is caused by a simple mechanical friction of the leaves.

Most of the commercially-grown varieties of cane in Cuba are shown to be susceptible to the attacks of the Pokkah-bong disease but the consequent loss is small. The POJ 2878 variety appears to be the most severely affected of any of the varieties under observation.

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PLATE I.

Plant affected with Pokkah-bong, showing the red markings on the chlorotic bases of the young leaves.

L.C.C. KRIEGER, PINX.

A. HOEN & CO., LITH.

UNIV. OF MICH.

RHIZIDIOCYSTIS ANANASI SIDERIS, NOV. GEN. ET SP., A ROOT HAIR PARASITE OF PINEAPPLES[1]

C. P. SIDERIS

INTRODUCTION

Rhizidiocystis ananasi is one of the many organisms that infest pineapple roots and are more or less responsible for the development of the disease known as "pineapple wilt." This organism is apparently new to science, since no mention of it can be found in the available literature.

The organism attacks and kills the root hairs of pineapple plants. It does not invade any of the other exodermal cells or the interior tissues of the root. Parasitism is definitely restricted to the root hairs proper. One may find the mycelium covering the very tip of the root but, even in such cases, the organism has never been observed to invade other cells except those of young and tender root hairs that are still in the process of development in the region of the root tip. There is no rot associated with infected tissues; and the death of the main root tissues, which sometimes occurs, is due to secondary causes.

TECHNIQUE

Owing to the fact that the organism is a strict parasite and not culturable on synthetic media, its isolation from infected fields required a special technique. Repeated attempts to grow it on sterilized pineapple roots that had been grown in water cultures and all of whose root hairs were intact, but dead, failed; the only recourse left was to grow it on living root hairs and study it there. The best method for conducting such studies was found to be by means of root-study boxes. Such boxes were used quite extensively by Dr. J. F. Illingworth, of the Entomology Department, Experiment Station of the Association of Hawaiian Pineapple Canners, and the organism was first observed in one of his boxes. These root-study boxes, known otherwise as root cages, were first introduced by Comstock (4) and later were used by Illingworth (8) for the study of the behavior of insects in the soil. The original plans for the boxes have been considerably modified by various members of this Station staff to meet the requirements of specific problems. Some of the boxes are illustrated in figures 1, 2, and 3. They may be made in any size for specific purposes, but the size most often used for these studies measures $16 \times 14 \times 2\frac{1}{2}$ inches. The essential feature is to have two glass sides through which the growth and development of the roots of the plant growing therein may be observed microscopically without the least

[1] Technical paper No. 6 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

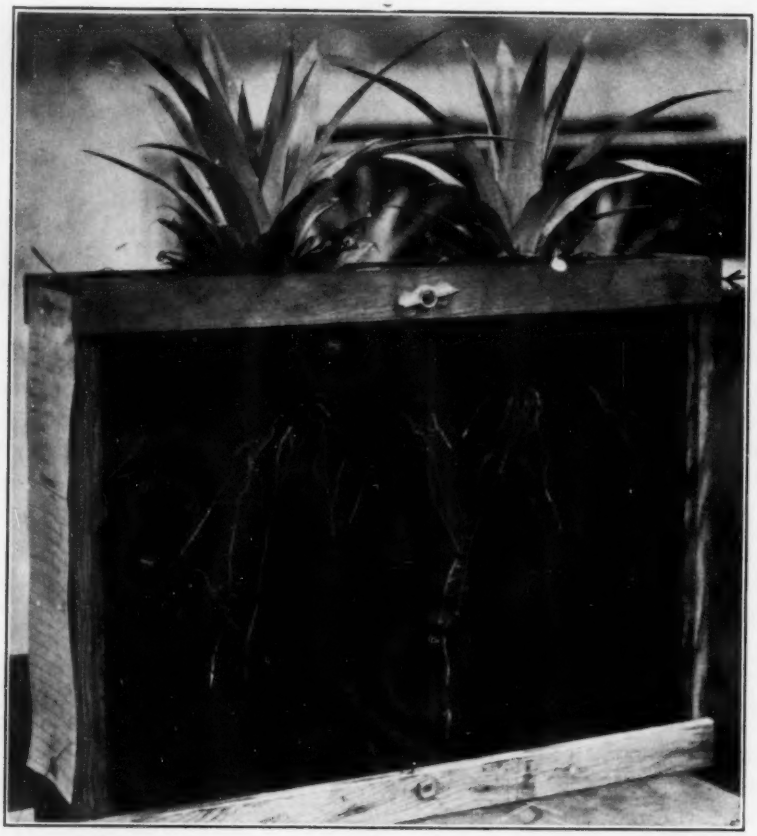

FIG. 1. A Comstock type root study box. In the more recent models hooks are being used in the place of the screws marked with an arrow. The wooden panel that is placed over the glass to keep the sunlight out has been removed in this case to show the roots as they appear against the glass side.

disturbance to the plant. Except during observation periods the glass sides are covered with wooden blinds. Both animal and vegetable organisms that live near or directly on the roots may likewise be observed and their relationships studied. The boxes are so constructed that the glass sides can be removed by releasing a hook without greatly disturbing the soil or the roots of the growing plant. Frequent microscopic examinations also are possible, as well as removal of diseased roots without injury to nearby roots or disturbance of the soil around them.

ISOLATION OF THE FUNGUS

The method used for the isolation of the fungus from various field soils was as follows: The soil under consideration was placed in a root-study

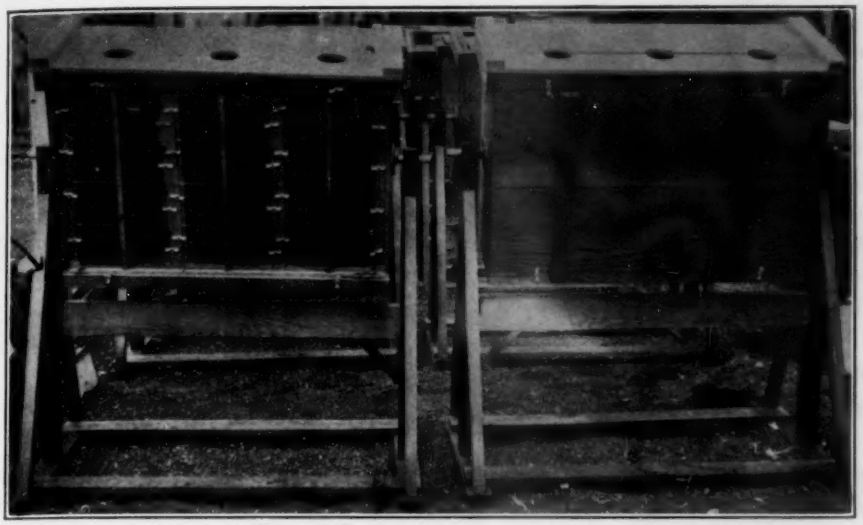

FIG. 2. Two root study boxes of the swinging type, one open and the other closed. In this model, measuring $1 \times 2 \times 3$ feet, plants can be grown for a longer time than in the smaller boxes. The arrow indicates the position of a hook by means of which the box can be fastened at any angle for microscopical observations.

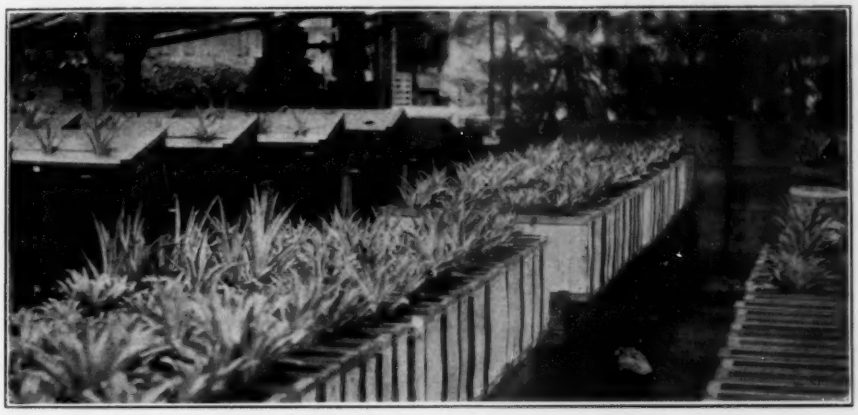

FIG. 3. Batteries of small and large root study boxes as they appear in the greenhouses of the Experiment Station of the Association of Hawaiian Pineapple Canners.

box, and one or two pineapple crowns that had been previously dipped in some fungicidal solution were planted. The growth and development of the roots of these crowns may be followed as closely as necessary with a microscope of the Greenough type mounted on a Dermatoscope stand. The fungus, if present, appears as a whitish cottony mass filling the air spaces

between the root hairs. Its filamentous hyphae are quite long and touch at widely separated points, thus presenting a more or less tangled appearance among the root hairs. Intercalary or terminal swellings are often present. The former are turbinate cells (Sammelzellen), and the latter zoosporangia and hypnospores (Dauersporen) of the organism.

Pure, or rather approximately pure, cultures of the organism may be prepared as follows: Transfers of extremely small pieces of roots infected with this fungus may be made to roots of other pineapple plants grown in root-study boxes containing sterilized soil. Owing to the rapid growth of this fungus on young pineapple roots new transfers may be made within 48–72 hours before any of the other organisms are able to reach the regions of the root already occupied by *Rhizidiocystis*. A number of successive transfers made in this manner yield an approximately pure culture of the organism on roots and in the soil. Soil inoculated in this manner may be used for the inoculation of other sterile soil in studies of this type.

In order to study the morphology and behavior of the organism, clean specimens are of paramount importance. As it is difficult to wash adhering soil particles from the root without destroying the root hairs and the organism thereon, it is easier and preferable to grow clean roots. This may be done as follows: The root box may be filled either with sterile river sand free from colloidal particles or with soil, the former being preferable. After the plants have produced roots about 1 in. long the sides of the root box are opened and furrows are made with a sterile spatula or other such object starting from the tip of the root and terminating at the bottom of the box. Furrows should be made in the direction likely to be followed by the root and should measure about ½ in. in thickness and depth. Extreme care should be taken in watering these boxes to avoid washing the surface soil downwards and filling up the furrows.

MORPHOLOGY OF THE ORGANISM

It has been quite difficult to identify this organism owing to its failure to grow on artificial media or on other natural media except living pineapple root hairs. Repeated attempts to grow it on the root hairs of pineapple plants grown in water cultures failed, notwithstanding the fact that the root hairs there were living and in good condition. The apparent cause for the failure of the organism to grow in the latter case is the excess water of the culture.

The results obtained from the root-box studies on the morphology of the fungus may be summarized as follows: (*1*) The organism produces abundant white mycelium packed in the air spaces between the root hairs of the plant (Fig. 4). The mycelium is evanescent, and almost immediately

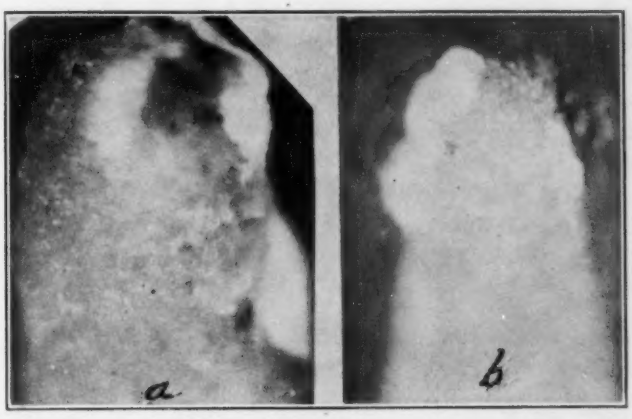

FIG. 4. Pineapple roots (×30) showing the white cottony mycelium of the fungus.

collapses and is no longer visible when the root upon which it is growing is cut off and removed from the soil atmosphere. (*2*) The hyphae are extremely fine and long, arachnoid in appearance (Fig. 6, *a* and *b*) with intercalary swellings (Sammelzellen) of spherical, triangular, oblong, or various other shapes and of various sizes (Fig. 6, *c*). The thin filamentous hyphae measure about 1 μ in diameter whereas the swellings may be from 3 to 6 μ. These swellings, technically known as turbinate cells, give rise to 1–8 branches, depending mainly upon their size. The protoplasm aggregates in the turbinate cells leaving the thin filaments empty. (*3*) The branches that are being produced from the turbinate cells produce at their extreme termini kidney-shaped zoosporangia (Fig. 5 and Fig. 6, *a* and *b*). These have never as yet been observed to give rise to zoospores. The zoosporangium when fully developed produces on its concave side an emission collar (Fig. 6, *a* and Fig. 7, *a*, *f*, *g*, *i*, *j*, *k* and *o*) from 1 to 2 μ in diameter and from 5 to 15 μ, or more, long. The zoosporangial emission collar serves the purpose of an appressorium to a certain extent besides that of discharging the protoplasmic contents that never develop into zoospores. The tube or emission collar enters the root hair of the pineapple plant (Fig. 8) wherein it discharges its protoplasmic contents which grow at the expense of the root hair, killing and assimilating the protoplasmic contents of the latter. (*4*) A mature zoosporangium gives rise to a hypnospore or resting spore, which is produced on the convex side of the zoosporangium (Fig. 9). The hypnospore starts as a tube, the end of which gradually enlarges until it becomes a sphere (Fig. 9, *a* to *h*). After it has reached full growth its surface becomes rough, the roughness increasing with age (Fig. 9, *i* to *o*). It happens occasionally that more than one hypnospore develops from the same zoosporangium; they may be produced either in

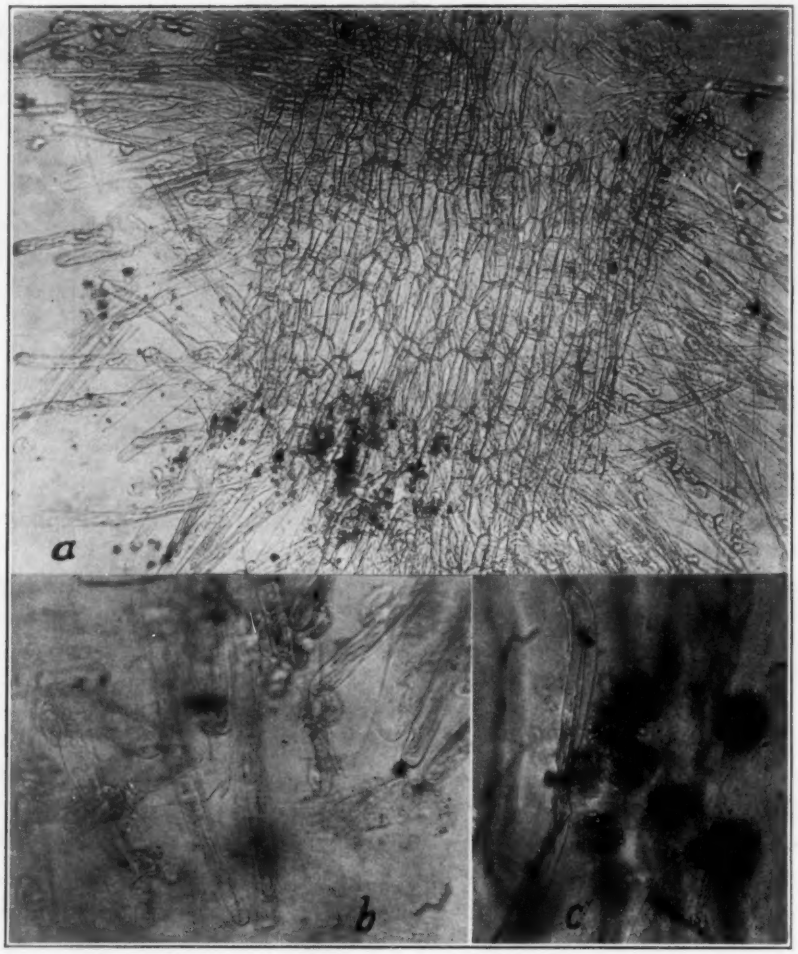

FIG. 5. (*a*) Portion of a pineapple root showing the zoosporangia of ***Rhizidiocystis ananasi*** attached to root hairs (×150). (*b and c*) Zoosporangia and hypnospores of the fungus attached to root hairs (×300).

chains (Fig. 7, *a* and *b*) or from two different tubes (Fig. 7, *c*, *d* and *h*). Not more than one of such hypnospores ever reaches maturity. Whether the hypnospore in this case represents the product of an asexual or sexual reproduction is not definitely known. The writer has observed quite often morphologically undifferentiated hyphae attached to such structures (Fig. 7, *j*, *k*, *l*, *m* and *n*) but, owing to the extreme minuteness of these hyphae, their relationship or union could not be definitely established. The details in the germination of hypnospores have not been fully investigated. It is

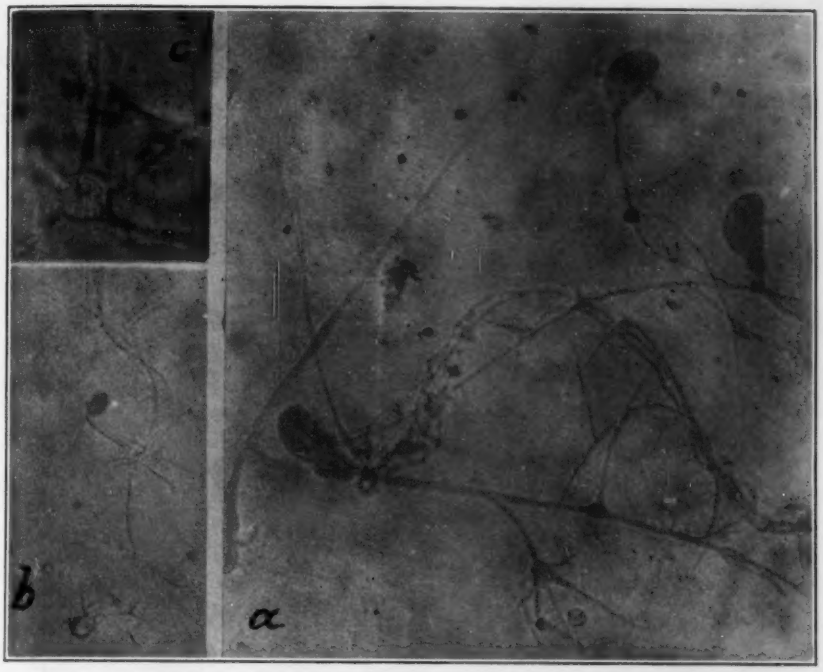

FIG. 6. (*a*) Mycelium, showing turbinate cells of different shapes and sizes and zoosporangia of *R. ananasi* (×600). (*b*) A young zoosporangium (×300). (*c*) A spherical turbinate cell with six branches (×1050).

certain that under the conditions of study the hypnospores did not produce zoospores, but in one or two cases the writer observed a flattened band or large hypha instead, broader at the base and pointed at the other end, which end extended but slightly beyond the rough shell of the hypnospore. This structure is filled with protoplasm and may be constricted at more or less regular intervals: turbinate cells (Sammelzellen) are formed at the constrictions. The hyphae that are produced on the turbinate cells and produce the zoosporangia are formed as soon as the turbinate cells are differentiated. As all these structures develop more or less simultaneously, the shell of the hypnospore becomes entangled in the newly produced mycelium, thus rendering extremely difficult the detailed examination of hypnospore germination. A germinating hypnospore seems to be between three and six times the size of one that is still attached to the zoosporangium. This is probably due to the fact that the shell of the hypnospore after cracking and opening occupies a greater area and therefore looks larger under the microscope. As hypnospores do not germinate at all seasons of the year, this fact alone increases the difficulties for their study. Germinating and germinated hypnospores have only been observed during the spring months, particularly

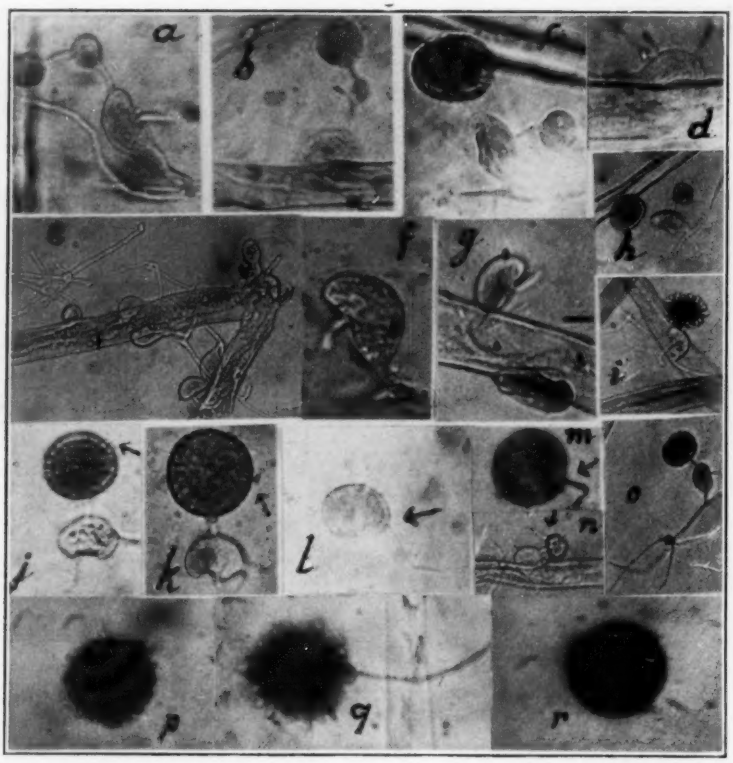

FIG. 7. (*a* and *b*) Zoosporangia with catenulate hypnospores (×600). (*c*, *d*, and *h*) Zoosporangia with two hypnospores (×600). (*e*) Root hair attacked by five zoosporangia (×300). (*f*) Zoosporangium (×1050). (*g*) Zoosporangium with emission collar turned upward (×600). (*i* and *o*) Zoosporangia with emission collar suspended in the air (×300). (*j*, *k*, *l*, *m*, *n*, *p*, and *q*) Hypnospores showing the point at which hyphae, morphologically nondifferentiated, may have fertilized them (*n*, ×300—others, ×600). (*r*) Hypnospore without its echinate shell (×600).

in April and May, and at temperatures ranging between 28° and 34° C. It is possible that they may germinate during the summer and autumn months but it is doubtful, as their germination has not yet been observed during such seasons.

CLASSIFICATION

The organism belongs, on the basis of its morphology, in the order Chytridiales and family Cladochytriaceae of the class Phycomycetes. As none of the known genera of this family manifests close similarity or identity in some of the striking features of this organism, the new genus

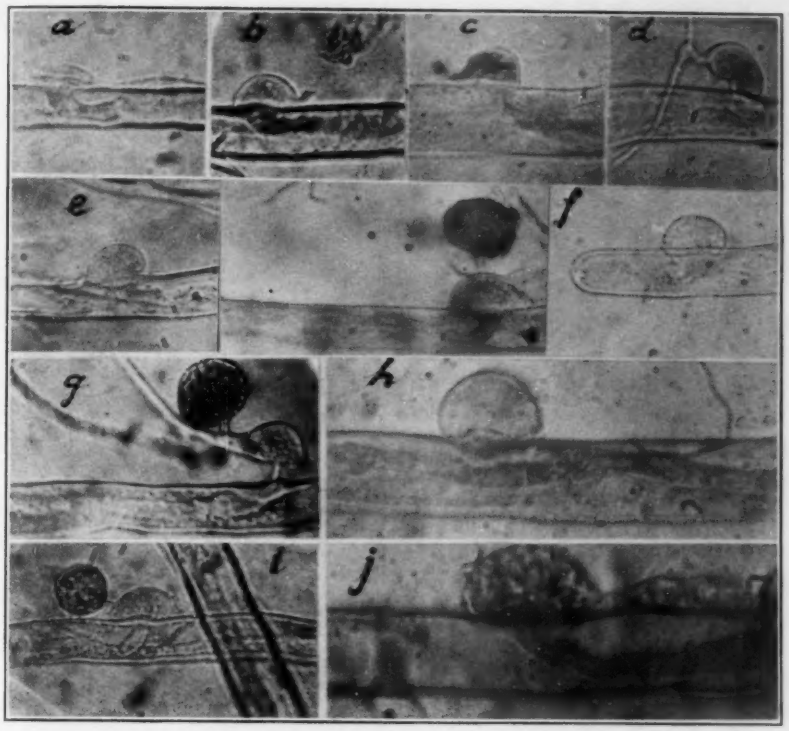

FIG. 8. (*a* and *b*) Thickened portion of the emission collar of the zoosporangium of *Rhizidiocystis* in a root hair (×600). (*c* to *j*) Zoosporangia attached to root hairs (*h* and *j*, ×1050; all others, ×600).

Rhizidiocystis[2] (from the Greek *rhizidion* = root hair and *cystis* = bladder) has been created.

[2] This organism has been recorded previously in Pineapple News (12) under the name *Nephromyces rhizidiophthoreus* Sid. The author believed at that time the generic name *Nephromyces* had never been used before in mycological nomenclature after having consulted Clements, "The Genera of Fungi"; Engler and Prantl, "Natürliche Pflanzenfamilien"; Wm. Englemann, 1897; Stevens, "The Fungi Which Cause Plant Disease"; and a few other works. After the name *Nephromyces* had been published the writer was able to secure a copy of Rhabenhorst's Kryptogamenflora; Fischer, "Die Pilze," IV Abt., Phycomycetes (5). There, on page 145 and under the title "Ungenau bekannte und sweifelhafte Gattungen der Hyphochytriaceae," the generic name *Nephromyces* Giard appears. The original paper (7) was immediately consulted and it was found that although the organism described by Giard as *Nephromyces* has some relationship to *Rhizidiocystis*, it represents, however, an entirely different organism from that studied by the present writer. Giard gave the name *Nephromyces*, meaning kidney fungus, to his organism because it was found in the kidney of certain ascidian species, whereas the present author assigned the same name on account of the kidney-shaped zoosporangia of the fungus.

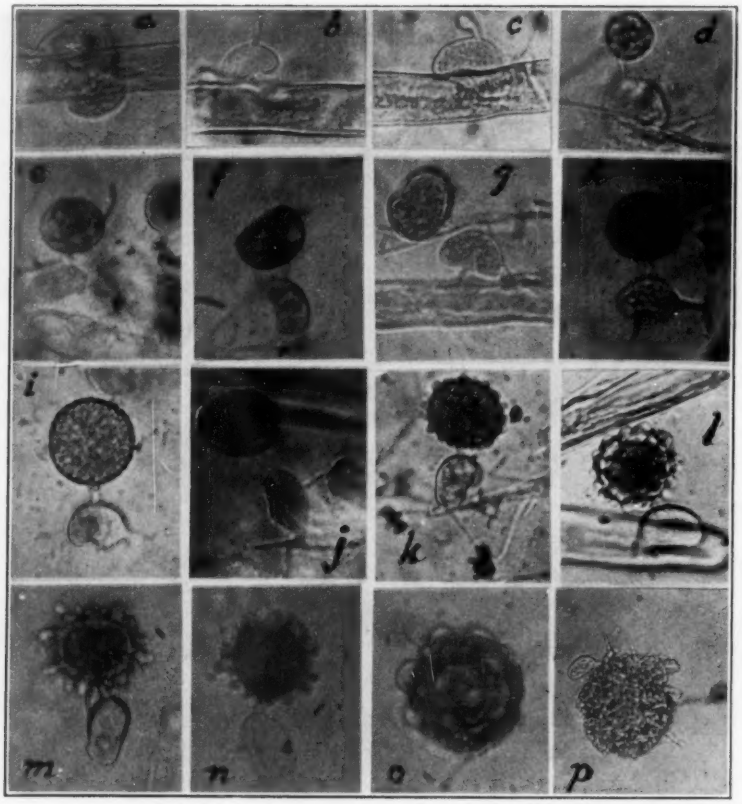

FIG. 9. (*a* to *n*) Different stages in the development of hypnospores (×600). (*o*) Mature hypnospore (×1050). (*p*) Germinating hypnospore not showing clearly on account of the roughness of the shell and mycelium surrounding it (×300).

Rhizidiocystis nov. gen.

Mycelium white and evanescent, composed of slender filamentous hyphae and turbinate cells of various shapes and sizes; filamentous hyphae 1 μ, or slightly more, in diameter; turbinate cells spherical, triangular, or irregular in shape, varying from 1 to 6 μ in diameter, and bearing from one to eight hyphal branches; kidney-shaped zoosporangia borne on the termini of the hyphal branches from the turbinate cells; zoospores are not produced, but the protoplasmic contents of the zoosporangium are discharged directly into the host cell through an emission tube, which is produced on the concave side of the zoosporangium as that organ matures; hypnospores are produced on the convex side of the zoosporangia, appearing as small tubes in the early stages, and later forming smooth spherical or ellipsoidal structures, which become rough and echinate as the hypnospores mature; hypnospores germinate to produce mycelium but never zoospores.

Rhizidiocystis ananasi nov. sp.

Mycelium white and evanescent, packed in the air spaces between the root hairs of the host plant; hyphae very long and slender, about 1 μ in diameter, extending from one root hair to another like spider webs; turbinate cells with one to eight hyphal branches; kidney-shaped zoosporangia, about 16 μ in length and 8 μ in diameter, produced at the ends of hyphal branches from the turbinate cells, and attached to the root hairs by means of an emission tube or suspended in the growing medium; emission tube, produced on concave side of zoosporangium, penetrates the root-hair wall and serves as means for the discharge of the contents of zoosporangium in a plasmodial state; the plasmodial mass grows within the root hair and kills and assimilates its protoplasm; hypnospores produced on concave side of zoosporangia, about 20 μ in diameter, smooth and tubular in the very early stages, spherical or slightly ellipsoidal and echinate in the later stages.

A strict parasite, which has been found only on living root hairs of pineapple grown in a natural soil habitat.

COMPARATIVE MORPHOLOGY OF RHIZIDIOCYSTIS AND OTHER MEMBERS OF CLADOCHYTRIACEAE

According to various taxonomists the outstanding characters that are more or less common to all members of the Cladochytriaceae are: (*1*) Very thin, filamentous, and evanescent mycelium; (*2*) turbinate cells (Sammelzellen) which vary in size and shape; (*3*) reproduction by means of either hypnospores or zoospores, or both; (*4*) sexual reproduction not definitely established, although it is possible that hypnospores may result from the union of male and female gametes.

Different investigators differ in their opinions of the phylogenetic position of this family. Bessey (1) considers it related to the Saprolegniaceae, its structural degeneration probably having resulted from hysterophytism and having affected the sexual reproductive organs more than the vegetative filaments. Gaümann (6) and Petersen (11), on the other hand, are of the opinion that Cladochytriaceae represent an ascending and not a descending group of organisms, their mycelium being better developed and more specialized than in the other families of Chytridiales.

There are quite appreciable morphological as well as physiological differences between the different organisms of this family, which complicate to a considerable degree their relationship and classification.

Cladochytrium (3, 10), which is the type specimen of the family, has very thin, filamentous hyphae from 1 to 2 μ in diameter and produces turbinate cells (Sammelzellen) which may become zoosporangia. The zoosporangia may produce zoospores which are liberated through a tube-like emis-

sion collar or may germinate directly with germ tubes instead of producing zoospores. *Amoebochytrium rhizidioides* Zopf (15), which is transitional between the Rhizideae and Cladochytriaceae, reproduces by aflagellate zoospores liberated from flask-like swellings with a tubular emission collar.

Nowakowskiella (2), *Physoderma* (14), and *Urophlyctis* (9) reproduce by zoospores or by hypnospores.

Rhizidiocystis behaves more like *Cladochytrium* than any of the other genera in the development and behavior of its zoosporangium; the zoosporangium germinates and its contents enter the tissues of the host directly without the formation of zoospores. The hypnospores (Dauersporen) of *Rhizidiocystis* are morphologically different from those of the other genera, being rough and to a certain extent echinate. The writer is of the opinion that the nonproduction of zoospores by the zoosporangia of *Rhizidiocystis* is due to change of habitat, namely, changing from an aquatic to a terrestrial mode of life, a condition forced upon it probably by its parasitic behavior.

PARASITIC BEHAVIOR OF RHIZIDIOCYSTIS

Rhizidiocystis ananasi is a parasite which is restricted to pineapple root hairs. It may be found quite often on the root tip where root hairs have not yet developed, but even in such cases it is superficial and has never been observed to invade the cells of this region. It is difficult to explain this high degree of specialization of the fungus. The root hairs are invaded by the emission collar of the zoosporangium, none of the other structures of the fungus entering the host tissues. It seems that these emission collars are highly chemotropic and it is possible that they locate root hairs in this manner. The angle formed by the emission collar with the zoosporangial wall is not always the same; angles varying between 45 and 90 degrees may be considered normal, but those that are as small as 15 and as large as 165 degrees are produced by movement of the emission collar in respect to a root hair which has either been invaded or is to be invaded. Emission collars have been observed to turn not only upwards and downwards in locating the root hairs of the host but also sidewise. All these tropisms of emission collars cannot be due to any single factor other than chemotaxis. Thigmotaxis may be excluded in this case on the ground that tissues other than root hairs are not invaded.

Whether the emission collar makes its way into the root hair by means of cytolytic enzymes or mechanical pressure has not yet been definitely established. After the root-hair wall has been penetrated, the emission collar enlarges in thickness at the point of entrance and immediately behind the root hair wall, forming a node, which tends to increase the holdfast of the organism on the root hair (Fig. 8, *a*, *b* and *i*). The writer has not been

able to ascertain the conditions that produce the enlargement of the fungal hypha at the point of entrance, that is, whether it is due to thickening in the wall of the collar or to an accumulation of protoplasmic contents. The zoosporangial contents then continue to grow inside the root hair by killing and gradually assimilating the protoplasmic contents of the host.

Rhizidiocystis ananasi makes a very prolific growth and is capable of attacking every root hair that has been produced or is in the process of development. Roots are literally denuded of their root hairs by this organism when grown in soils favoring its optimum development. It is true that there is no rot or destruction of the main root tissues. The root, however, grows slenderer as the destruction of its root hairs continues until it becomes atrophied and may persist in that condition until it dries up and dies, or is attacked by other fungi and destroyed. Roots that have become infected by *Rhizidiocystis ananasi* die very slowly; this is especially characteristic of plants growing in soils of low moisture content. With soils of high moisture content the damage is not so pronounced, as the roots grow more rapidly and consequently have more root hairs than the fungus can kill in a given period. It has been observed that with rapidly growing varieties of pineapples the damage to the root system caused by *Rhizidiocystis* is not so great as with slowly growing varieties (13). The reason is that the organism is not producing zoosporangia as fast as the roots are producing root hairs and as a result many of the root hairs are able to escape destruction. Old root hairs that may be dead have never been found invaded by *Rhizidiocystis ananasi*; it is invariably young root hairs that are invaded and killed. Inoculation studies with *Rhizidiocystis ananasi* have shown that practically all varieties and strains of pineapples are susceptible.

DISTRIBUTION

The organism is more or less restricted to pineapple fields. Repeated attempts were made to recover it from forest land, sugar cane fields, banana land, virgin land, and from cultivated land on which pineapples had never been grown; but none of the attempts were successful. In land that was planted to pineapples recently or within the last ten years, the presence of the fungus was detected in the majority of cases. Soil was obtained from a field that was planted to pineapples 20 years ago but never since then, and there the fungus was not found. The organism has been isolated from small as well as large fields, from fields that are situated in the center of the main pineapple districts, as well as from isolated fields. The latter are represented by such fields as the Kapalama substation, Mr. Meyer's field on the island of Molokai, Mr. Gay's field on the island of Lanai, and the Pearl City Fruit Company's fields in the Waianae district. Soil

samples were secured from areas inside and outside these fields; in the former case the organism was isolated, whereas results in the latter were negative. All the studies conducted to date indicate that the organism is intimately associated with pineapple plants; in all probability its other hosts, if any, must be exceedingly few or rare. Inoculation studies conducted on *Saccharum officinarum*, varieties Lahaina, Yellow Caledonia, D–1135, and H–109, kindly furnished by Mr. C. C. Barnum, of the Hawaiian Sugar Planters' Association Experiment Station, gave negative results. Other plants such as *Cajanus indicus*, variety New Era, Panicum (*Pennisetum barbinode*), and sisal (*Agave sisalana*) likewise gave negative results, indicating that the organism is not carried by any one of these plants that often precede the pineapple plant in the field.

That this organism occurs outside of the Hawaiian Islands is not improbable. If we assume that the pineapple plant is the only host of this organism, the latter must have been introduced into the Hawaiian Islands together with pineapple plants sometime in the past. If, on the other hand, the organism has more than one host, then again it is probable that the organism exists in other parts of the world where its hosts are distributed, unless such hosts are restricted to indigenous Hawaiian plants not growing elsewhere.

Its distribution to other parts of the world from the Hawaiian Islands is quite probable, as various countries have imported planting material grown in the Hawaiian Islands.

PROPHYLAXIS AND CONTROL

As it is difficult to combat the fungus once it has invaded a field, every effort should be made to prevent its introduction. There are a number of common-sense agricultural practices that are at the same time intimately connected with field sanitation. Before and after a field is planted the agricultural implements used for the purpose should be kept away from infested fields and as free as possible from accidental contamination. The planting material to be used should be taken from a field that has been kept free from infection and should be sterilized by some fungicide to safeguard against accidental contamination. The free movement of implements, animals and other carriers should be either prohibited or strictly supervised and all chances for dissemination of the infection be eliminated.

In cases of fields already infested with the fungus the best procedure for freeing them from the infestation is by means of dry fallow for some years, the extent depending upon the climate, physical properties of the soil, and other such conditions. Before an infested field is planted again to pineapples a test should be made for the presence or absence of *Rhizidiocystis ananasi* in the soil by means of root-study boxes.

Not knowing what other plants may serve as hosts of this fungus, it is unwise to recommend the eradication of certain plants and not of others. Volunteer pineapple plants, however, should under all conditions be destroyed.

SUMMARY

Rhizidiocystis ananasi nov. gen. et sp., a new member of the family Cladochytriaceae, order of Chytridiales, has been isolated from pineapple roots where it attacks and kills the root hairs. It is a strict parasite, not growing on any other living tissue of the pineapple plant or on any other plants that have been studied, nor on natural or synthetic bacteriological media.

It may, under favorable conditions, destroy all of the root hairs of a plant, produce atrophy of the main root tissues and cause a gradual drying up and death of the root or roots. There is no rot produced in the tissues of the root by *Rhizidiocystis ananasi* so long as such root or roots are kept free from secondary infection.

Rhizidiocystis reproduces by means of hypnospores. Whether these organs are gamic or agamic has not been definitely settled. The germination of hypnospores has not been studied in great detail owing to many complicating factors.

The zoosporangia of this organism have never been observed to produce zoospores. The emission collar of the zoosporangium penetrates the root hairs of the host, wherein it discharges its contents, which kill and gradually assimilate the protoplasmic contents of the root hair.

The hypnospore (Dauerspore) is produced on the convex side of the zoosporangium as a tube and later assumes the form of a smooth sphere. In later stages of its development, the sphere becomes rough and more or less spiny. Hypnospores do not germinate readily and under all conditions.

Turbinate cells (Sammelzellen), a common feature of all genera of the family Cladochytriaceae, are produced in abundance in various shapes and sizes. The hyphae are slender, arachnoid, filamentous, and evanescent.

The organism has not been reported outside of Hawaii, although is believed that it may occur wherever pineapples are grown.

The writer is indebted to Doctors A. L. Dean, J. F. Illingworth, G. H. Godfrey, and Mr. G. E. Paxton for their kind cooperation throughout these studies.

EXPERIMENT STATION,
ASSOCIATION OF HAWAIIAN PINEAPPLE CANNERS,
HONOLULU, HAWAII.

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THE DEVELOPMENT OF SOIL ROT OF TOMATOES DURING TRANSIT AND MARKETING

G. B. Ramsey and Alice Allen Bailey

The southern or winter-grown tomatoes, produced for marketing in the North during the winter and early spring months, frequently suffer great damage due to decay caused by the common soil fungus, Rhizoctonia. The disease is especially serious following heavy rains and is so obviously associated with wet soil conditions that the significant common name "soil rot" has been almost universally applied to describe this particular decay. The causal organism, *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.), appears to be present in all of the important trucking regions of the South. The amount of damage caused in any one season is entirely dependent upon weather conditions.

Rosenbaum[1] found soil rot causing considerable damage to Florida tomatoes during transit in 1918, and showed that the causal organism could spread through the wrapping from one fruit to another, thus causing decay of the neighboring stock, provided the fungus found entrance through a wound or through the stem scar. About this same time, Link and Gardner,[2] working on the market diseases of vegetables, reported that soil rot of tomatoes, "appearing as it does in shipments from almost all regions and especially the South and California, probably caused more losses in the market than any other single factor."

During the past few years, Weber and Ramsey[3] have found soil rot especially destructive in the low muck soil of Florida. They report that when wet and moderately cool weather prevails during February and March this disease often destroys a very high percentage of the fruit. Usually only those tomatoes touching the soil or hanging near enough to become spattered with water and soil during rains are affected. Only occasionally fruits hanging well up in the vine develop soil rot. For this reason staked tomatoes are much less subject to infection than those not staked.

Observations and studies made by the writers during the past eight years on the important receiving markets of the North show that in almost every shipping season the tomatoes from Florida, Texas, Mississippi, California,

[1] Rosenbaum, J. The origin and spread of tomato fruit rots in transit. Phytopath. **8**: 572–580. 1918.

[2] Link, Geo. K. K., and Max Gardner. Market pathology and market diseases of vegetables. Phytopath. **9**: 497–520. 1919.

[3] Weber, G. F., and G. B. Ramsey. Tomato diseases in Florida. Fla. Agr. Exp. Sta. Bul. 185: 61–138. 1926.

and Mexico are subject to more or less serious loss from soil rot. Inspection certificates issued by the Food Products Inspection Service of the United States Department of Agriculture show that cars of tomatoes having from 5 to 15 per cent of decay due to soil rot are not uncommon during wet seasons, and occasionally the decay may range as high as 60 per cent. A random selection was made of certificates covering 26 cars of Florida tomatoes that showed soil rot when inspected in the northern markets during the spring of 1926. In these cars there was an average loss of over 7 per cent due to this disease. In the best car there was an average of 2 per cent decay, while in the poorest the range was 15 to 50 per cent in the individual crates, the average for the car being 35 per cent.

These data show that, under favorable conditions, soil rot is one of the most destructive tomato diseases affecting southern stock. In view of its importance it seemed desirable to make a study of this disease as it occurs during transit and in the market, as well as in the fields and packing houses. The results herein reported cover field and packing-house investigations in Florida, Texas, and Mississippi; test shipments from these States; and laboratory and market studies made at Chicago from 1926 to 1928.

The early infections in the field are characterized by small circular brown spots on the lower half of the fruit. Usually the spots which are one-fourth inch in diameter and larger show narrow alternating zones of light and dark brown tissue which form definite concentric rings (Fig. 1, A). This is characteristic of spots up to one inch or more in diameter on green fruit in the field but, during transit, as the tomatoes ripen and the fungus makes more rapid growth, these concentric rings are obliterated (Fig. 1, B). On the market, soil rot appears on the fruit as large, brownish or reddish brown lesions (Fig. 1, C). In some instances, however, the lesion has a muddy-brown cast due to the retention of part of the green color in the decayed area as the fruit ripens. Cream-colored or light-brown mycelium is often visible on the surface of the tomatoes when they arrive on the market, and may penetrate the moist wrappings and form irregular brown sclerotia on them as well as on the fruit. Frequently the paper wrapping becomes firmly attached to the tomato at the place where such mycelial mats occur (Fig. 1, D). The decay produced is firm at first, but becomes softer and more watersoaked in appearance as the fruit ripens and the fungus penetrates deeper into the tissues. In the larger lesions the epidermis ruptures and the crack becomes filled with brown mycelium and sclerotia.

The early symptoms become visible on the fruit in the field about the third day following a rain. So long as the top soil is fairly dry Rhizoctonia does not come to the surface or cause appreciable trouble, even though many of the fruits may be in contact with the soil. Where the plants are

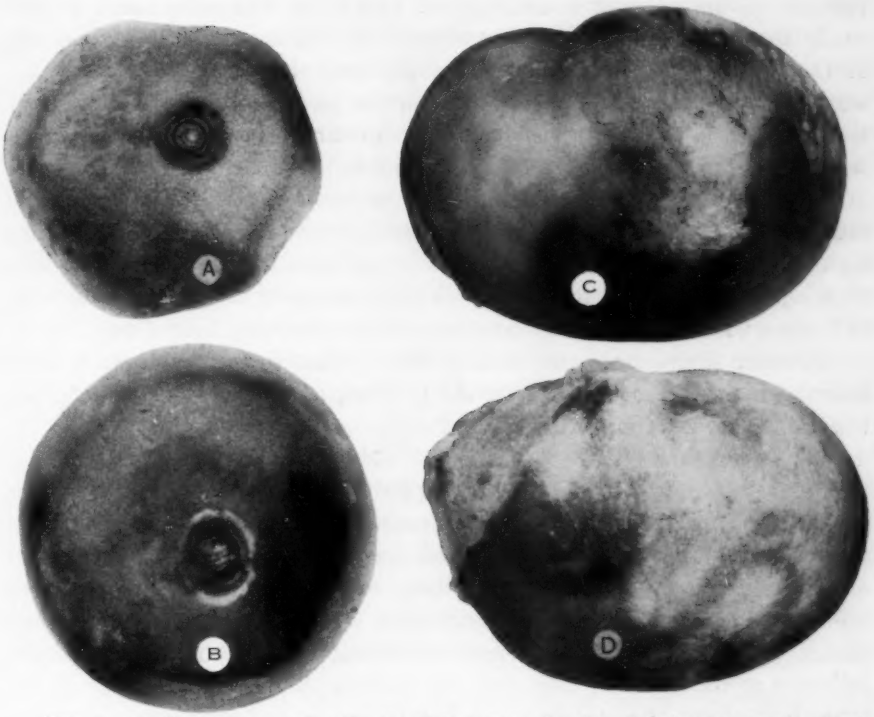

FIG. 1. A, Early stage of soil rot of tomatoes showing definite concentric rings. B, A later stage showing development of lesion and obliteration of rings. C, Soil rot as it appears in the receiving market. D, Advanced stage showing surface mycelium and part of moist wrapping attached to the fruit.

large and spreading and the foliage is dense enough to hold the soil moisture and maintain a high degree of humidity underneath the vines, soil rot may occasionally be found for long periods following rain. This is especially true in localities where heavy dews which drench the plants are common. Under ordinary conditions, however, most of the affected tomatoes decay, and within a week or ten days following the rainy period have disappeared.

Most of the important commercial growers of tomatoes recognize the danger from soil rot and take precautions to see that little affected stock is sent to the packing house. Following heavy rains during the packing season, the laborers are instructed to discard the decaying tomatoes in the field. Those showing large lesions are eliminated, but in many instances a rather high percentage of affected tomatoes reaches the packing house. For example, in April, 1926, at Goulds, Fla., a careful inspection of one grower's stock showed 30 per cent soil-rot infection of fruit in the field boxes

brought to the packing house. After this stock was sorted and graded ready for packing, a reinspection showed that there was still 18 per cent of the graded fruits affected by this disease. In other words, approximately 12 per cent of the stock reaching the packing house showed lesions large enough to be plainly visible and was discarded in the commercial sorting and grading, whereas the remaining lesions were so small that they were not seen, or at least not considered likely to cause decay during transit and marketing. It is these small lesions, however, which cause so much loss during marketing. In some instances it has been found necessary to stop packing tomatoes for a day or two in order to allow the decay to develop to a stage which can be easily recognized and sorted out before packing.

In order to determine the rate of development in transit of these small lesions which were overlooked by the graders, as well as some larger ones, test shipments were made to Chicago.

EXPERIMENTAL SHIPMENTS

In selecting tomatoes for experimental shipments, precautions were taken to exclude all fruit that showed mechanical injury or diseases that might influence the rate of development of soil rot during transit. All tomatoes were mature, green commercial stock and were wrapped and packed in six-basket crates or lugs, according to the commercial practice followed at the point of origin of the various shipments. Because of the rapid breakdown of tomatoes which had lesions 20 mm. or more in diameter when packed, only those with lesions covering a range in size below 20 mm. were selected for shipping. The diameters of all spots were measured in millimeters and the sizes marked on the tomato with India ink. On arrival at Chicago the tomatoes were unpacked and the spots remeasured in order to determine the amount of development during transit. In this manner, accurate data were obtained on the rate of development of lesions of various sizes. The percentage of new lesions developed in transit was noted, and their average diameter also obtained. For the purpose of determining the amount of spread in transit, healthy tomatoes and infected fruits were mixed in the experimental shipments.

For detailed study, special consideration is given to 522 tomatoes that were included in experimental shipments. Of these, 168 showed soil-rot lesions when packed at the shipping point, while the remaining 354 healthy fruits were interspersed among the diseased stock. Seven of these infected fruits and 44 of the healthy ones, or 9.8 per cent of those shipped, developed new soil-rot lesions during transit. There was a total of 337 soil-rot lesions present on the 212 affected tomatoes when they arrived at Chicago, 82 of which were new lesions. On arrival, 9.7 per cent of the new lesions

and 12.7 per cent of those present on shipping, were contaminated, most of them with bacteria or Rhizopus, and one with Melanconium.

All but 12 of the new lesions (14.6 per cent) developed in transit as a result of contact with the wet papers of infected fruit. Approximately one-half of the new lesions occurred where there was no evident break in the skin. In 16 per cent of the infections a wound or bruise was the place of entry, in 25 per cent of the cases the organism entered through the stem scar, and in 7.4 per cent through the blossom scar. Besides the 70 tomatoes which showed new lesions due to spread in transit 55 were covered with mycelium on arrival but showed no lesions, and the wrappings of 22 more were moist and overgrown with mycelium and sclerotia. These 77 fruits were replaced in their wrappers and held for a while at room temperature. In four days, 6 of them developed Rhizoctonia lesions under the mycelium on the surface of the fruit, and 2 developed lesions which were contaminated with bacteria. Three additional pure soil-rot lesions developed in 13 days. The new lesions developed on both green and ripe fruit in transit and storage. These data indicate that Rhizoctonia can enter the tomato fruit through an apparently sound epidermis, if in contact with it long enough. Field observations lead to the same conclusion.

In table 1, the increase in diameters of soil-rot lesions of various sizes during transit and the average daily increases in diameter are shown. For

TABLE 1.—*The increase in diameters of soil-rot lesions on tomato fruits during transit*

Diam. of lesion at shipping point (mm.)	No. days in transit	Diam. of lesion at end of transit period (mm.)	Increase in diam. of lesion (mm.)	Average daily increase in diam. of lesion (mm.)
0	5	11.6	11.6	2.3
	7	24.7	24.7	3.5
1–5	5	17.2	13.2	2.6
	7	29.9	25.8	3.7
6–10	5	30.8	22.5	4.5
	7	43.3	35.6	5.1
11–15	5	42.0	29.8	6.0
	7	55.2	42.6	6.1
16–20	5	47.0	29.0	5.8
	7	60.0	42.3	6.0

convenience, the spots were considered in five groups, the 0 representing the new spots that developed during the transit period. From this table, it will be seen that the rate of development was much more rapid in the larger

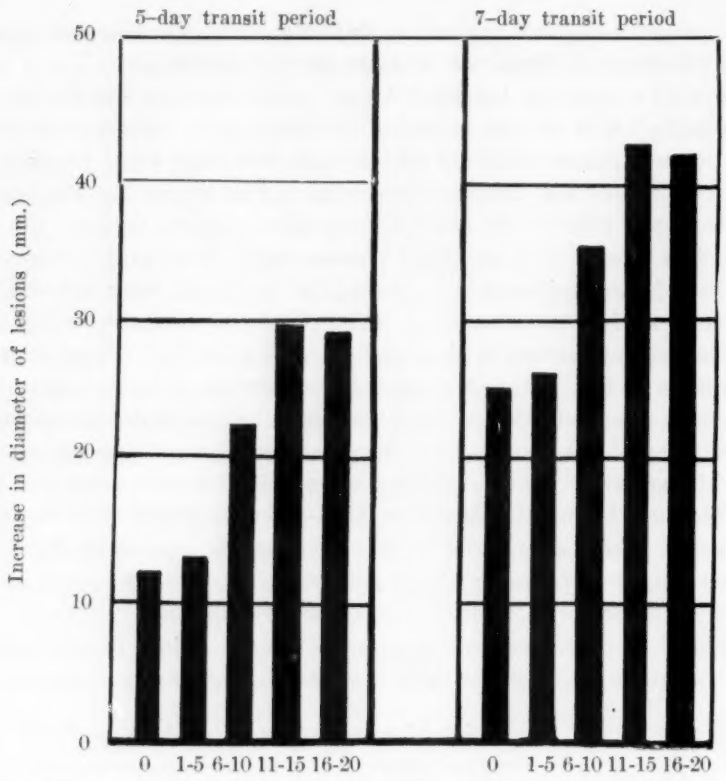

FIG. 2. Average total increase in size of soil-rot lesions on tomato fruits during transit.

spots than in the smaller. The maximum rate was attained in spots approximately 15 mm. in diameter. Spots of this size enlarged about twice as fast as those 5 mm. or less in diameter when packed. The slowest of these, however, developed rapidly enough to destroy the marketability of all affected stock, as shown in figure 2, which shows graphically the average total increase in size for 5- and 7-day transit periods. A transit period of 5 days would be considered reasonable for most shipping points in the States under consideration, yet in this moderate time the new spots enlarged to 11.6 mm. in diameter (Table 1). The visible spots up to 5 mm. in diameter, which constitute the majority of those overlooked in commercial grading and packing, attained an average diameter of 17.2 mm. by the time they reached the market after 5 days in transit. However, the fact that spots 30–40 mm. in diameter are found on tomatoes on arrival at the market indicates that spots larger than 5 mm. are sometimes included in commercial shipments.

INOCULATION EXPERIMENT

The following data obtained from artificially infected fruit give further evidence of the rapid development of soil rot in transit. In all test shipments of naturally infected tomatoes, the lesions were for the most part 5 mm. or larger in diameter. In order to determine the rate of development when infection through wounds takes place just previous to packing, one lug of inoculated fruits was sent to Chicago from Texas.

Seventy-three mature green tomatoes, selected for freedom from disease, were inoculated by inserting a small bit of mycelium into a slight scalpel wound made on the side of each fruit. These tomatoes were kept at a temperature of 75°–80° F. for 15 hours before they were wrapped and packed. At the end of this time, slight discoloration and decay were visible along the edges of the inoculation wounds. On arrival at Chicago after five days in transit, an examination showed all of the tomatoes to be seriously affected with soil rot. The lesions found varied from 7 to 43 mm. in diameter, and from 5 to 20 mm. in depth. The average diameter of all spots was 20.4 mm. After the spots were remeasured, the tomatoes were again wrapped and packed in the lug and kept for two days at a temperature of 66°–72° F. On examination this time, the average diameter of all spots was 38.1 mm., and the decay ranged from 20 to 40 mm. deep.

SUMMARY

1. Soil rot is caused by the common soil fungus, *Rhizoctonia solani* Kühn, which appears to be present in all of the southern trucking regions.

2. This disease is sometimes responsible for great losses in tomato shipments during transit and marketing, as well as in the field and packing houses.

3. The amount of damage is directly related to weather conditions. Heavy rains or successive moderate rains that keep the surface soil wet favor the development of soil rot, whereas, under moderately dry conditions where the surface soil is dry, the disease is of no economic importance.

4. Experimental shipments of southern tomatoes made during 1926–27 and 1927–28, averaged 9.8 per cent new lesions on arrival at Chicago. About 50 per cent of these lesions developed in both green and ripe fruits which showed no break in the epidermis. In the rest of the cases, the organism entered through wounds and stem and blossom scars. The new lesions averaged 11.6 mm. in diameter after five days in transit.

5. The rate of development of soil-rot lesions varied directly with the size of the spots, the maximum rate occurring in those approximately 15 mm. in diameter when the shipment started.

6. The visible spots up to 5 mm. in diameter, which constitute the majority of those overlooked in commercial grading and packing, attained an average diameter of 17.2 mm. in five days transit.

7. A shipment of inoculated tomatoes showed 100 per cent infection. The lesions produced averaged 20.4 mm. in diameter and ranged from 5 to 20 mm. deep at the end of five days in transit.

OFFICE OF VEGETABLE AND FORAGE DISEASES,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE.

THE OCCURRENCE OF SCLEROSPORA GRAMINICOLA ON MAIZE IN WISCONSIN

WM. H. WESTON, JR.[1]

In November, 1925, Melhus and Van Haltern (3), at the Iowa Agricultural Experiment Station, reported that they had successfully inoculated seedlings of corn artificially with *Sclerospora graminicola* (Sacc.) Schroet. from the green foxtail grass, *Setaria viridis* (L.) Beauv. After describing the effect of the disease on the inoculated maize plants and noting that the production of conidiophores and conidia occurs in the case of corn only in the seedling stage and is much less even at its maximum than the ordinary sporulation on *Setaria viridis*, they state: "This probably explains why no one has recorded the presence of this disease [*i.e.*, on corn] before."

In view of the interest which any such record necessarily would deserve it seemed desirable, even after a lapse of some years, to report an earlier case of the occurrence of this disease on corn. Accordingly, in the summer of 1928, the writer prepared a manuscript embodying these records and also the conclusions arrived at from his own study of the diseased material from Wisconsin. Before this manuscript could be put into type, however, there appeared the detailed publication on this subject by Melhus and his associates (5) following their previous brief abstract (4). These publications make an extended discussion of the matter unnecessary, but it is believed that the historical value of the following paragraphs still warrants their publication.

On July 8, 1921, R. B. Streets, while studying at the Field Laboratory of the Department of Plant Pathology, of the University of Wisconsin, near Sturgeon Bay, Door County, Wis., collected material of the conidial stage of a downy mildew on leaves of young Golden Bantam sweet corn on the farm of S. T. Learned. As the conidiophores and conidia when examined under the microscope were obviously those of the genus Sclerospora, two possibilities suggested themselves; first, that the fungus might be one of the four or five Oriental conidial species of Sclerospora so severely destructive to maize, which, in some way, had been introduced into the United States and was starting to develop and spread on corn; or second, that the fungus might be the widely distributed *Sclerospora graminicola* common in that

[1] Formerly Pathologist in Charge of Downy Mildew Investigations, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, who expresses his thanks to Dr. C. R. Ball and Dr. H. B. Humphrey, of that Office, for kindly placing at his disposal progress reports, correspondence, and other material relating to the subject of this note.

region on *Setaria viridis* and from this grass, transmitted by chance, under conditions unusually favorable for infection, to occasional maize plants near by.

A preliminary study of this material by Dr. Otto Reinking and G. A. Ocfemia, who had known the destructive Philippine downy mildew of maize in the Philippine Islands, led them to believe that, in all probability, the fungus was not that species of Sclerospora. As the writer, then Pathologist in Charge of Downy Mildew Investigations in the Office of Cereal Crops and Diseases, of the Bureau of Plant Industry, had recently returned from two years of study of the Sclerosporas of maize and related crops in the Philippines, Dr. E. M. Gilbert sent to him, also, some material which he, after examination and comparison, agreed was certainly not the destructive Oriental species. This material will be more fully discussed later.

FIELD SURVEYS AND EXPERIMENTS IN WISCONSIN

The infection noted by Mr. Streets did not spread to other plants of maize but gradually disappeared after he had collected nearly all of the leaves of the plants examined which showed the sporulation of the fungus. Further study of the situation seemed highly desirable, however, both to establish the identity of the fungus and to determine whether it would spread and maintain itself on maize. Accordingly, Mr. Streets, in the summer of 1922, was engaged to study further the situation in Wisconsin and at the close of his work he made a report to the Office of Cereal Crops and Diseases, the main results of which may be outlined as follows:

Beginning about the middle of August he carried on a field survey in the vicinity of Madison, Wis., in order to see if the same transmission of the causal organism might be taking place from the Setaria so commonly infected by *Sclerospora graminicola* thereabouts, but found none of the fungus on either host at that time. Proceeding to Sturgeon Bay on August 24, Mr. Streets stayed until the middle of September, making a very careful field survey and also attempting natural inoculations under field conditions. At that time, sweet corn again had been planted in part of the same plot where he had found Sclerospora on corn the preceding year and in this field, as Mr. Streets reported, "the plot was examined plant by plant on two different occasions but no trace of Sclerospora was found on the corn. Adjacent fields within a radius of one-half mile were all scouted two rows at a time by examining every stunted or abnormal plant; more extensive scouting was done also in different parts of the peninsula." "In brief, my best efforts failed to find the fungus on corn at this season (1922), when, according to prevailing weather conditions, it should have been fruiting abundantly if it had survived the dry part of the summer in an active condition."

In addition to this extensive survey, Mr. Streets made attempts to secure infection by natural inoculation in the field from *Setaria viridis* to maize. On August 25, for example, as there was too little conidial material on Setaria for experiment, the oospore-containing tissue of host plants was "spread on the ground along the rows of corn (recently planted Golden Bantam) which had not yet emerged." The young corn plants grew up through this material but no visible infection had occurred by September 11, when Mr. Streets made his last inspection, nor by September 30 when examined by others.

Mr. Streets made a second survey of the district in the summer of 1923, working somewhat earlier in the season when the conditions were better for the transmission of the fungus to corn than they had been in the latter part of the previous summer. In July, he intensively surveyed many fields around Madison, Wis., especially those of sweet corn, but found no sign of infection by the downy mildew, anywhere, even on such especially favorable fields as some of the college experimental plots which were planted to varieties of sweet corn and had numerous plants of Setaria infected by the downy mildew near by. The weather was hot and dry, however, and even on the Setaria there was very little spore formation by the fungus.

Proceeding to Sturgeon Bay in the latter part of July, 1923, Mr. Streets continued an intensive survey in the same locality where he had found the infection on maize in 1921. The situation was better than in the previous summer of 1922, as the weather was more favorable and the Sclerospora was as abundant as ever on the Setaria. Yet even in the field where the original infection had been found, and where Golden Bantam was again planted in three sections of different ages, the youngest being then about six inches high, no infection by the downy mildew was found. The survey was extended into August and through a considerable part of the surrounding territory so that no portion of the county was overlooked, but no cases of infection were found on maize.

Mr. Streets also made attempts at this time to secure natural inoculation in the field. In July, 1923, for example, he planted heavily infected plants of Setaria very close to young Golden Bantam corn so that the leaves intermingled with those of the corn plants of different ages. The weather was not particularly favorable for natural infection, however, so no infection resulted, and some of the transplanted Setaria died. In August, this attempt was repeated under much more favorable weather conditions when there were several periods of considerable rainfall and high humidity favorable to infection and when abundant new lesions were appearing on Setaria itself. Yet no infection of maize was secured in this manner. Early in August, also, attempts were made to infect young maize plants artificially

by placing conidia from Setaria in drops of moisture on the leaves and by spraying the conidia on to the maize from an atomizer, subsequently protecting the inoculated plants by covering them with inverted fruit jars and shading them from direct sunlight. Not one of these attempts was successful.

The work just reported, even though largely negative, did bring out the following points. The species of Sclerospora found on maize at Sturgeon Bay in the summer of 1921 did not spread further on that host during that summer nor did it appear again on that host in that locality in the summers of 1922 and 1923. Moreover, no other cases of infection by this downy mildew on this host were encountered in that whole county nor around Madison during those two summers.

STUDY OF INFECTED MAIZE FROM WISCONSIN

Mention was made previously of the specimens of Golden Bantam sweet corn sent from Wisconsin to the writer by Dr. E. M. Gilbert. The material consisted of a few small pieces of dried blades of leaves, somewhat discolored but showing occasional narrow linear elongate streaks, probably once etiolated but now somewhat brownish, on which here and there on the lower leaf surface a close, rather granular, grayish to buff colored down could be seen. In general these linear streaks were not so broad nor so extensive as those which characterize systemic infection of maize by the conidial Sclerosporas of the Orient; rather did they resemble those typical of the local infection spotting which the writer and G. F. Weber (11) found on the leaves of *Setaria* (*Chaetochloa*) *magna* in Florida. The down from the leaves, when carefully mounted, was found to consist of Sclerospora conidia and conidiophores in rather poor condition for comparative study; first, because they were obviously the last scanty remnants of the previous night's sporulation dried by the morning sun and collected during the day, and second, because they had become overrun with an Aspergillus by which the leaf specimens, probably through inadequate drying, had become moulded here and there.

On microscopic examination even this material showed clearly that the fungus was not one of the destructive conidial Sclerosporas attacking maize in the Orient. All of these species have relatively large conidiophores, with an extensive and elaborate branch system, and a definite basal cell or foot, while in this Wisconsin material the conidiophores were short and stocky with small, irregular branches and no clearly defined basal cell. The conidia also were quite distinct, for they were small in size and rather broadly ellipsoidal in shape, even smaller than those of the smallest Oriental species, *S. javanica* Palm (7) with its rather rotund conidia, the bulk of

which are 21 to 27 μ in length by 15 to 20 μ in diameter, and strikingly different from the much larger and more elongate conidia of the other Oriental species,—*S. maydis* (Rac.) Butl. (1), with spores mostly 28 to 45 μ long by 16 to 22 μ diameter; *S. sacchari* T. Miyake (2, 6), with spores mostly 35 to 43 μ long by 16 to 22 μ diameter; *S. philippinensis* Weston (8), with spores mostly 31 to 37 μ long by 15 to 20 μ diameter; and *S. spontanea* Weston (9), with spores mostly 39 to 45 μ long by 13 to 18 μ diameter. Even more distinctive in mature conidia of the Wisconsin material was the presence of a modified portion of the wall at the apex, the so-called papilla of dehiscence through which the zoospores would have emerged from these conidia (*i.e.*, zoosporangia) on germination, for the conidia of all the Oriental species just enumerated have true conidia which utterly lack any papilla of dehiscence and always germinate by means of germ tubes.

All of these characteristics of the Wisconsin material, namely, the short stocky conidiophores with their small irregular branch system and lack of any distinct basal cell, and the small broadly ellipsoidal conidia (zoosporangia) with the apical papilla of dehiscence, were very strong indications that the fungus was *Sclerospora graminicola* (Sacc.) Schroet. On May 22, 1922, after having examined the material, the writer wrote Dr. Gilbert that "The Sclerospora is not the typical Philippine species nor is it like the other forms in the Oriental tropics that have been described from Java, India, and Formosa. It most closely resembles *Sclerospora graminicola* which is the common parasite of Setaria (Chaetochloa) collected by J. J. Davis in your region." However, the caution attending an identification of poor material prompted adding "there are, however, points of difference which may mark it as distinct from that species but it is hard to be sure from dried material collected during the day time."

These differences were a seemingly greater irregularity of the branch system and apparently smaller size of the conidia, but later after going over these points in careful comparisons with material of *S. graminicola* on Setaria from Minnesota, North Dakota, and other localities in the United States, the writer became entirely convinced that these points of difference were not significant and that the fungus on maize in Door County, Wis., was in fact this species. Indeed, the size of the conidia in the Wisconsin material, in which the great proportion of the spores (for example, 72 out of 100) fell in length classes between 15 and 20.9 μ and in diameter classes between 11 and 14.9 μ (94 out of 100), was found to be quite in agreement with similar measurements of the species on Setaria in Minnesota gathered fresh during nocturnal sporulation but killed in Flemming's weaker solution and mounted in dilute glycerine, or with those of this species on Setaria in North Dakota mounted in water and then dilute glycerine after previous

moistening with alcohol, from herbarium specimens, that had been collected during the day and merely dried. Probably, as the writer later pointed out (10, p. 777), if measurements were made of conidia when shed at night in dew or other moisture under optimum conditions the modes of length and diameter would be increased by one 2 μ class of size, but as all of the material compared was fairly comparable in preparation the close agreement in size seems dependably significant.

Mr. Streets' discovery of *Sclerospora graminicola* on Golden Bantam sweet corn in Wisconsin in 1921 is of especial interest as the first recorded, though unpublished, account of the infection of this host by this parasite in nature. As the evidence presented by this one case of natural infection is in agreement with the evidence of the extensive artificial infections secured by Melhus and Van Haltern, it seems thoroughly justifiable to expect that from time to time cases of transmission of this species of Sclerospora to maize from near-by gramineous weeds will be encountered in nature elsewhere.

It is worthy of note in connection with the occurrence of *Sclerospora graminicola* on sweet corn in Wisconsin that in Iowa Melhus *et al.* (5, p. 331) observed *Sclerospora graminicola* occurring naturally on corn in the field only twice, once on dent corn seedlings at Boone in 1925, and once on Golden Bantam sweet corn nearing maturity at Story City in 1927. It also is interesting to note their statement that "A survey made in June, 1927, which included readings on 3,350 corn plants (dent, pop and sweet) located at 19 places in 16 of the north central counties of Iowa, revealed no positive symptoms of infection of Sclerospora."

LABORATORIES OF CRYPTOGAMIC BOTANY,
HARVARD UNIVERSITY.

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A SCALE FOR MEASURING AREAS OF RIBES LEAVES[1]

ROBERT S. CARUTHERS

In studying plants, occasions arise when it is necessary to determine the surface areas of their leaves. The planimeter method of measuring the areas of an extensive series of leaves is not only tedious, but also entirely unsuited to periodic measurements of growing leaves. When an accuracy of 95 per cent satisfies the purpose for which the measurements are undertaken, the planimeter method may be replaced by the use of "leaf-area scales" (Fig. 1), which can be applied without injury to growing leaves,

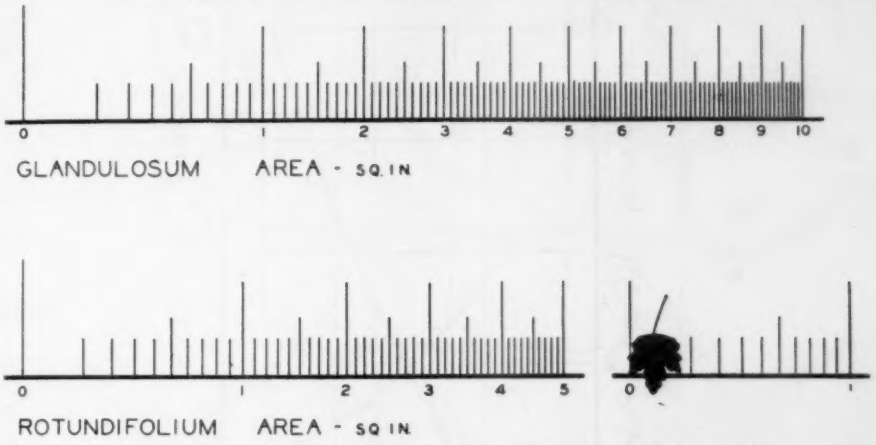

FIG. 1. Leaf area scales for *Ribes glandulosum* and *R. rotundifolium* constructed from logarithmic graphs of average areas in corresponding width classes, figures 4 and 5.

and by means of which areas can be read directly from one or two lineal dimensions. The construction of these scales is a simple matter. The scales illustrated in figure 1 were developed for determining the areas of leaves of currants and gooseberries (Ribes spp.) in connection with the experimental eradication of these plants to protect white pine from blister rust (*Cronartium ribicola*). The leaves measured for constructing the scales were collected from plants growing under average conditions of shade and moisture in different localities near North Hudson, New York.

As the leaves of a given species are usually somewhat similar in shape, general relations exist between their areas and lineal dimensions. In basing a leaf-area scale on one lineal dimension, greatest accuracy will result from the use of that dimension offering the greatest coefficient of variability. If

[1] The writer is indebted to Mr. G. B. Posey for direction and assistance in the preparation of this paper.

1 to 3 inches represents the range of lengths for normal leaves of a certain species and 1 to 4 inches the range of widths, obviously the width will have the greater influence on area and will furnish the longer, and therefore the more accurately readable scale.

A relation existing between the area and a transverse dimension of a leaf must make itself apparent in the measurements of the area and the given dimension for a representative sample of the leaves of the species. If a series of width measurements are plotted on regular cross-section paper against corresponding areas, and the points lie on a smooth curve, a relation exists between the area and width which is shown graphically by the shape of the curve and analytically by the equation of the curve. It may be said, how-

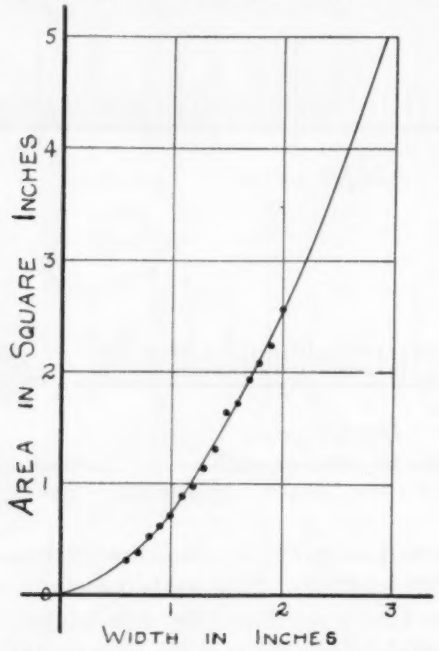

FIG. 2. Graph showing the relation between the areas and widths of leaves of *R. rotundifolium* plotted from the average area in each corresponding width class (Table 1).

ever, that abnormally shaped leaves are often of too frequent occurrence to allow a small sample of leaves to establish the average relation between a lineal dimension and the area. Even though a sufficiently large sample of leaves were measured to include all possible variations in leaf shape, weighted as to frequency of their occurrence, still it would be difficult to pass a curve through the plotted points which would represent the average relation. In most cases the points will be found to deviate too greatly from the curve to permit its accurate location without the use of detailed mathe-

matics. However, if an average area is determined from the measurements of 10 leaves for each tenth-of-an-inch width size class, the data will be condensed to represent more nearly average conditions. If these values are plotted on regular cross-section paper, a curve may be drawn by eye through the points. Such curves have been determined for two species, *Ribes rotundifolium* Michx. and *Ribes glandulosum* Grauer (Figs. 2 and 3). Average

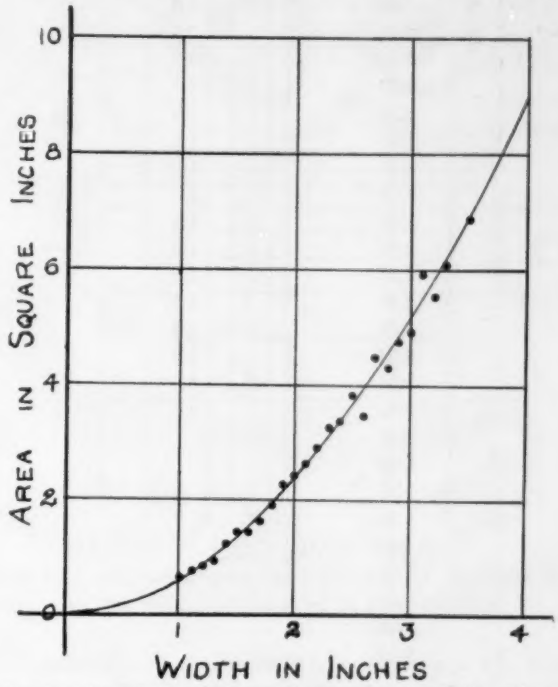

FIG. 3. Graph showing the relation between the areas and widths of leaves of *R. glandulosum* plotted from average area in corresponding width class (Table 1).

areas were determined for each tenth-of-an-inch width size class from 0.6 to 2.0 in. for *R. rotundifolium* and from 1.0 to 3.5 in. for *R. glandulosum* except for the 3.4-in. size, which was lacking. These measurements are tabulated in table 1.

The curves (Figs. 2 and 3) for *Ribes glandulosum* and *R. rotundifolium* are parabolic in form; therefore slight accuracy can be expected in their extrapolation on regular cross-section paper. It can readily be shown, however, that such curves on regular cross-section paper must assume a linear form when the same data are plotted logarithmically. In figures 4 and 5 the measurements in table 1 have been plotted on logarithmic cross-section paper, forming straight lines which may be extrapolated with great accuracy.

TABLE 1.—*Measurements of width and corresponding area for Ribes rotundifolium and Ribes glandulosum*

R. glandulosum		*R. rotundifolium*	
Width in inches (Class centers)	Area in sq. inches (Average of 10 measurements)	Width in inches (Class centers)	Area in sq. inches (Average of 10 measurements)
1.0	0.64	0.6	0.31
1.1	0.76	0.7	0.38
1.2	0.84	0.8	0.52
1.3	0.93	0.9	0.62
1.4	1.22	1.0	0.71
1.5	1.43	1.1	0.88
1.6	1.43	1.2	0.98
1.7	1.61	1.3	1.14
1.8	1.90	1.4	1.31
1.9	2.27	1.5	1.63
2.0	2.41	1.6	1.72
2.1	2.63	1.7	1.93
2.2	2.90	1.8	2.08
2.3	3.26	1.9	2.24
2.4	3.38	2.0	2.56
2.5	3.83		
2.6	3.47		
2.7	4.49		
2.8	4.30		
2.9	4.75		
3.0	4.94		
3.1	5.93		
3.2	5.54		
3.3	6.08		
3.5	6.87		

Not only does logarithmic plotting simplify extrapolation, but it also permits a great reduction in the amount of data required for scale construction. As only two points are needed to determine a straight line, average area values need to be determined for only two values of width, one in the upper and the other in the lower ranges of leaf sizes. There is a possibility, however, of introducing rather large errors if the two points determined deviate from the straight line which would result were area measurements determined for intermediate widths in the range of leaf sizes. Therefore the two points should be determined from a much larger group of leaves than when each intermediate point is determined. Twenty-five measurements for both the upper and lower limits of width size classes would in all probability locate the straight line with as great accuracy as though ten leaves were measured in each tenth-of-an-inch width size class.

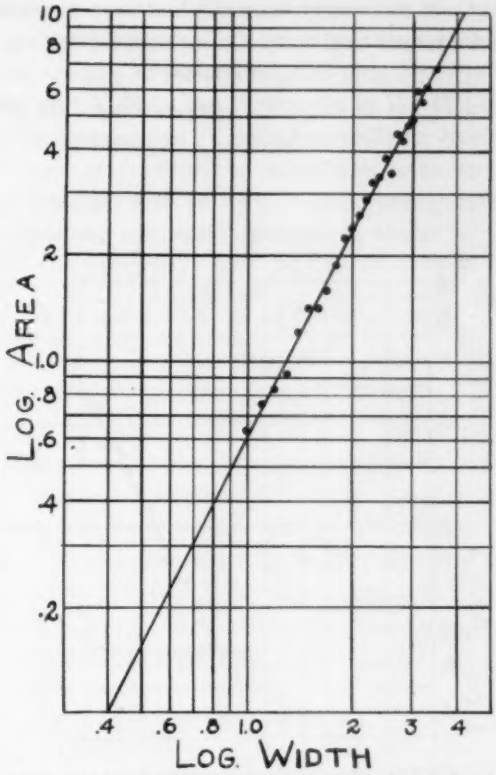

FIG. 4. Graph showing relation between the logarithms of the areas and the logarithms of the widths of leaves of *R. rotundifolium*.

The graduations of the scales shown in figures 1 are derived from the logarithmic graphs. Widths corresponding to each tenth of a square inch increment of area are laid off along a straight line. On the scale thus constructed the width graduations are marked with their corresponding area values. The area of any normal leaf of the species can then be determined as illustrated (Fig. 1).

The manner in which these leaf-area scales were constructed prevents them from being applied with accuracy to individual leaves or to short and non-representative series of leaves. If it is undesirable to use a planimeter in such cases, it is possible to increase the accuracy of the scale method by the construction of two scales instead of one, the second based upon another dimension of the leaf, such as length. Since irregularities in leaf shape frequently occur, as abnormal widths with subnormal lengths and vice versa, the area of an individual leaf may be closely approximated by averaging the readings of both scales.

The primary object in constructing the leaf area scales shown in figure 1 was to obtain instruments that would approximate within 10 per cent the area of large groups of leaves of each species of Ribes. Before putting the scales for *R. glandulosum* and for *R. rotundifolium* into general use, rough checks were obtained on their accuracy by comparing the scaled areas with the planimetered areas of new series of leaves.

The scale for *R. glandulosum* (Fig. 1) was checked by measuring the areas of 100 leaves taken at random from one large plant, by both the

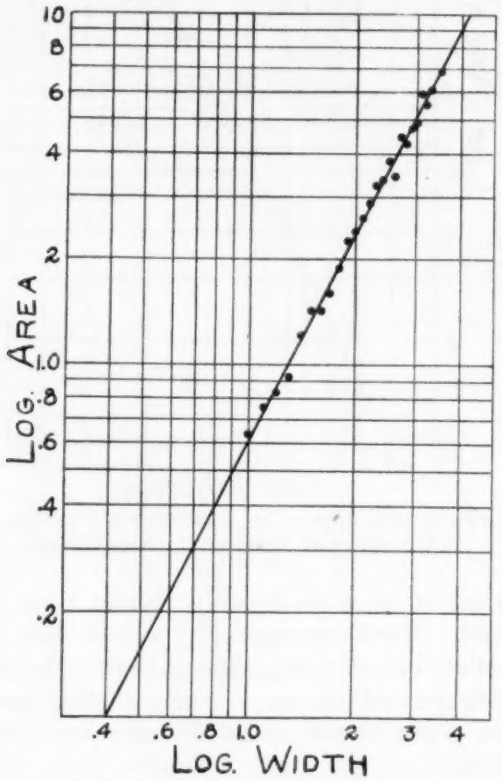

FIG. 5. Graph showing relation between the logarithms of the areas and the logarithms of the widths of leaves of *R. glandulosum*.

planimeter and scale methods applied independently by two individuals. The total area of the 100 leaves was found to be 275.6 sq. in. by both methods. In this check, 100 per cent relative accuracy is accidental and cannot be expected consistently.

The scale for *R. rotundifolium* (Fig. 1) was checked by measuring in like manner all leaves (a total of 605) not mutilated by insects from 10

plants. The total area of the 605 leaves measured by planimeter was 327.5 sq. in., and by the scale was 332.7 sq. in., a variation of 5.2 sq. in., or a plus error of 1.59 per cent by the scale method.

Judging from these somewhat meager tests, the two scales may be used with consistent accuracy greater than 95 per cent. However, the accuracy of any leaf-area scale will depend upon how well the species is represented in the sample of leaves from which the scale was constructed and in the sample being measured. Since a large sample of leaves is more likely to be representative of the species, it may be expected to furnish the more accurate results.

SUMMARY

This paper shows how to construct and test the accuracy of a scale for measuring the surface area of Ribes leaves. The scale reads directly in terms of area and gives results that are between 90 and 100 per cent accurate. A scale of greater accuracy can be constructed from a large series of leaf measurements. As the scale method of measuring the surface area of leaves is rapid, it will save much time in measuring a large number of leaves when an accuracy within 5 or 10 per cent is sufficient. This method should be valuable to phytopathologists and ecologists.

OFFICE OF BLISTER RUST CONTROL,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

ROOT-OBSERVATION BOXES

A. L. Dean[1]

Most of the ills to which the pineapple plant is heir—and it has rich legacies—are root troubles. Observation and study of growing roots in large numbers have become routine matters in our laboratories. This study of roots and their diseases has been greatly facilitated by the use of root-observation boxes. There is nothing especially new about the idea of using such boxes, but to use them so intensively and extensively in the study of roots is apparently unusual. With the thought that others may find them equally useful, this description is given.

Two types of boxes are being used. The small ones are modeled after those first described by Professor J. H. Comstock[2] in 1888 as "root cages." The large type was evolved here before we had any knowledge of the Comstock root cage. Both are useful, but for different purposes.

The small box is shown in figures 1 and 2. The exact dimensions are not matters of great importance; those given have proved convenient. These boxes are readily made in large numbers at very low cost. Briefly, they consist of a wooden frame comprising two ends and a bottom, with side strips top and bottom, and two glass sides. Wooden shutters are provided to keep the contents dark except at times of observation. With a table saw, the wooden pieces can be rapidly cut to size and saw scarfs run to receive the glass sides. A useful arrangement for removing the glass readily is indicated in figure 2. The upper side strips which hold the tops of the glasses in place are secured at one end by a single nail or screw and at the other by hook and eye. By unhooking the fastening and swinging the strip upward, the top of the glass is freed and the pane can be lifted out without disturbing roots or soil, and as readily replaced.

These boxes have found extensive use in biological field surveys of pineapple soils. Large numbers of soil samples have been placed in these boxes and pineapple slips or crowns set in them, as indicated in figure 1, and the bozes placed in the glass-house. In a short time the roots start out and numbers of them grow along the glass sides. Every few days the boxes are examined for signs of root injury. Examination is readily made by laying each in turn in an almost horizontal position on a stand built for the purpose, removing the shutters, and examining the roots through the glass, first

[1] Director, Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii, Honolulu, Hawaii.

[2] Cornell University. Bulletin of the Agricultural Experiment Station III; November, 1888.

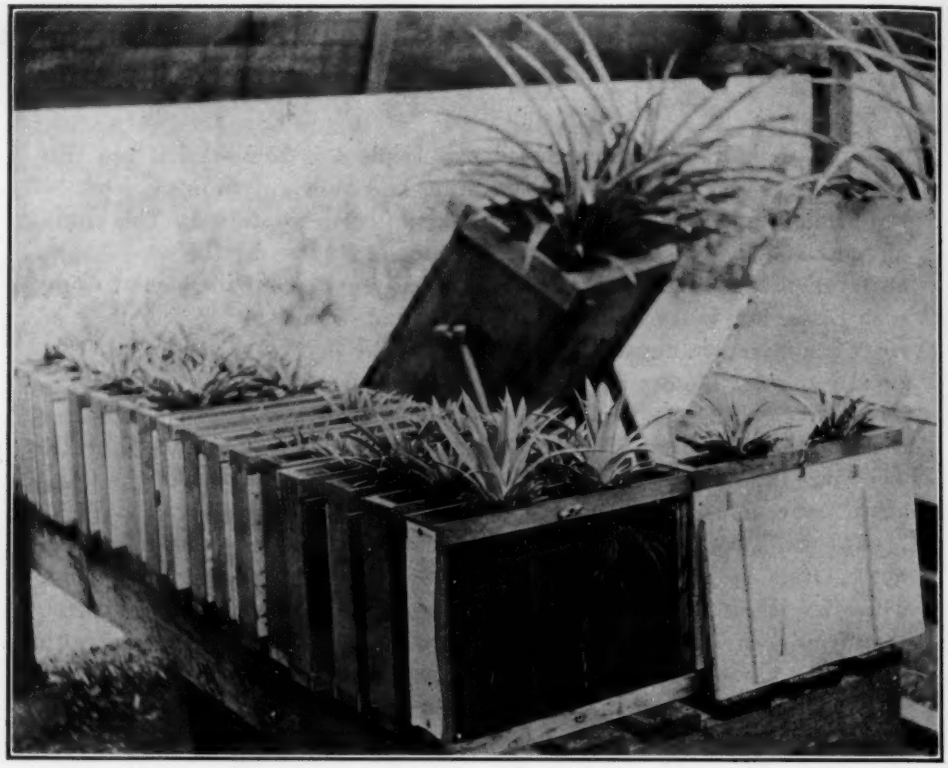

FIG. 1. Small root-observation boxes; a large box in the background.

with the unaided eye and then with the binocular. By examining both sides of a box a large number of roots is inspected and signs of injury readily detected. If portions of diseased roots are wanted for intensive study, they can readily be secured by carefully lifting the glass and cutting out desired portions. Soil insects may be observed at work, nematode injury followed, and the incidence and progress of fungus attacks noted. A good qualitative, and a rough quantitative, parasite analysis of a soil sample is thus secured. Parasites which would have wholly escaped us by the usual method of pulling up sickly plants in the field have been discovered at work and their progress observed in detail.

Studies of the life histories of various root parasites and of the extent and nature of the damage done have been facilitated. In a large steam sterilizer the boxes, with soil in place, are readily sterilized. Clean, healthy plants set in such sterile containers and soil will, when roots have started out, be in ideal condition for inoculation and subsequent study. By care-

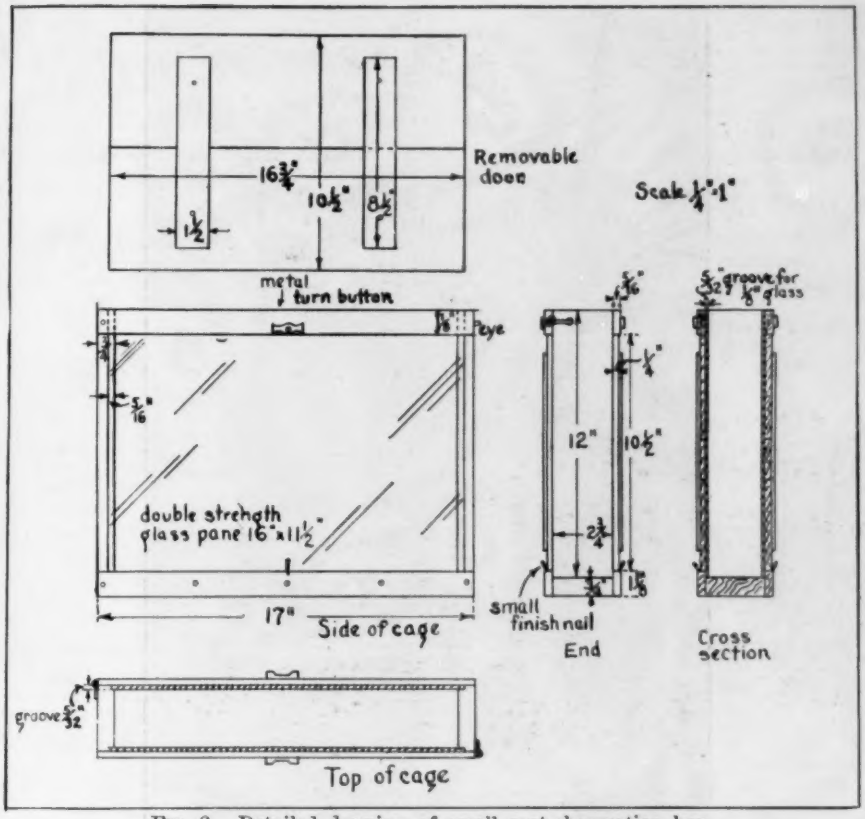

FIG. 2. Detailed drawing of small root-observation box.

fully removing the glass the inoculum may be placed in any desired position relative to the roots, the glass replaced, and observations made on the course of events. Effects of chemicals, temperature, moisture, etc., on host and parasite are easily observed.

It is obvious that physiological studies involving observations of roots are facilitated by the use of such glass-sided boxes. We have cemented strips of glass into these boxes with battery wax in such a manner that the space inside was divided into two portions by this glass partition reaching almost to the top. A plant growing astride of this partition sends part of its roots into each compartment, where different conditions may be provided.

The large type of box was developed here before we had become acquainted with the Comstock root cage. These boxes are shown in figures 3 and 4. They are adapted to studies involving more prolonged growth and

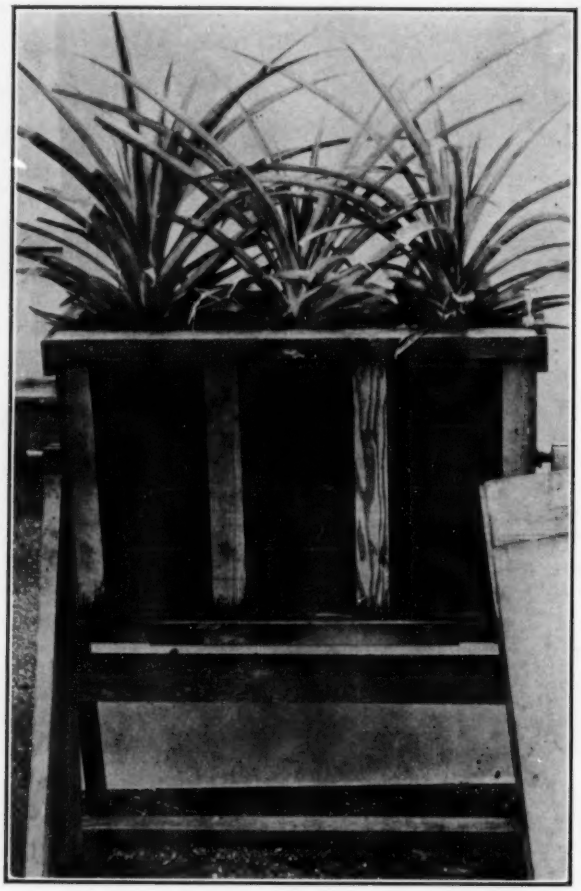

FIG. 3. Large root-observation box with shutter removed.

greater development. These larger boxes are provided with a number of panes of glass held in place by wooden strips in such a manner that the panes can be removed if desired. Wooden shutters are provided as in the small boxes. Our first boxes were made to stand on a bench. This was not convenient, however, when one wished to use the binocular microscope. As now made, the boxes have a ¾-inch pipe flange attached to each end and a short piece of pipe screwed into each. These pieces of pipe rest in slots in the supporting frame, allowing the boxes to swing. In this way the box may be swung up into a horizontal position and held there by a hook; or a stick can be used to support it if desired. The binocular can then be

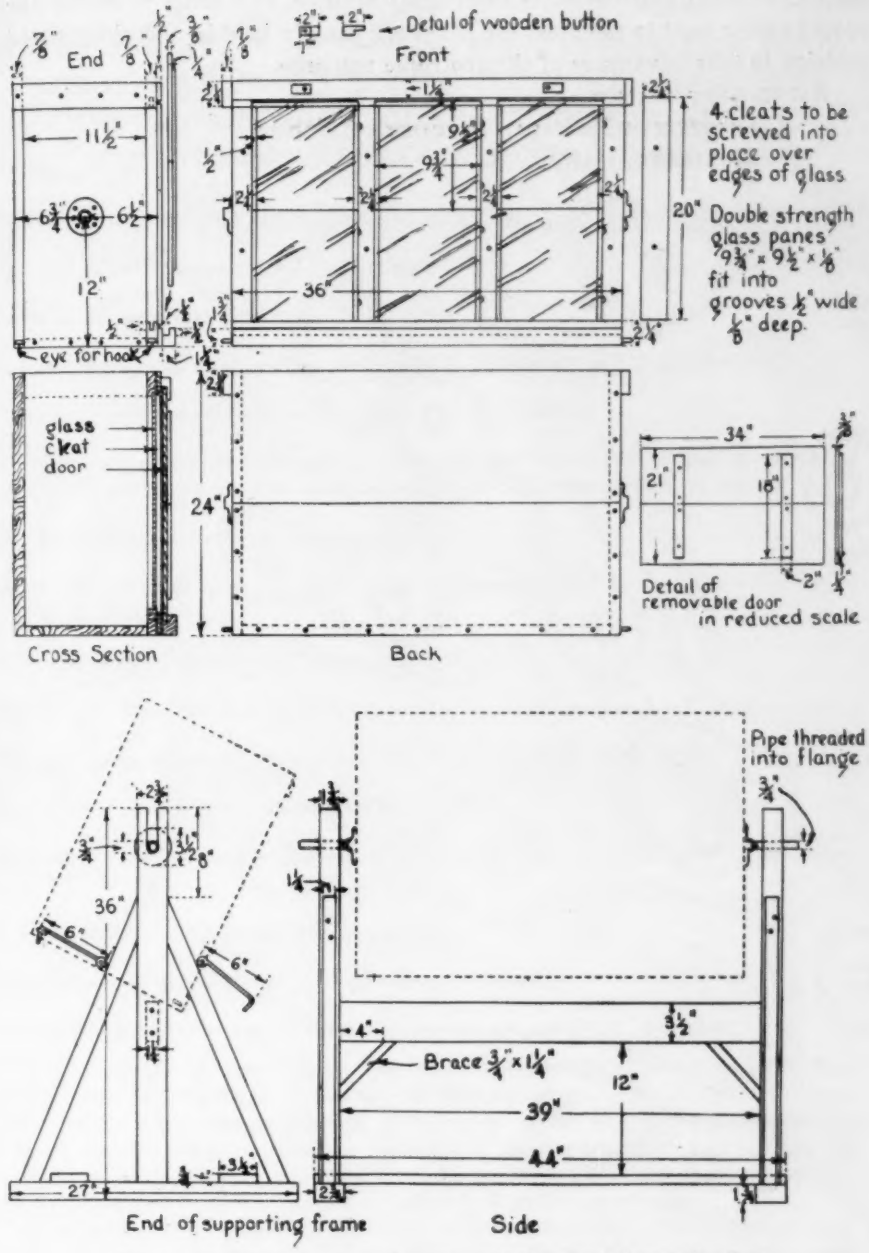

FIG. 4. Detailed drawing of large, swinging root-observation box.

used in comfort and the roots thoroughly studied. In order to encourage roots to grow next to the glass the boxes are usually held in a slightly tilted position to take advantage of the geotropic response.

EXPERIMENT STATION,
ASSOCIATION OF HAWAIIAN PINEAPPLE CANNERS,
HONOLULU, HAWAII.

TITLES AND ABSTRACTS OF PAPERS READ AT THE TENTH ANNUAL MEETING OF THE CANADIAN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, GUELPH, ONTARIO. DECEMBER 20 AND 21, 1928

Further experiments with millet smut.—R. E. STONE.

The spur blight of raspberries.—L. W. KOCH.

Notes on the fireblight situation.—D. H. JONES

Acid sterilization of liquid culture media.—B. G. MONTSERIN and J. G. COULSON.

President's address—Retrospect and Prospect.—D. L. BAILEY.

A preliminary report of the results of some field tests in the use of seed disinfectants for the prevention of loose smut of barley.—J. E. HOWITT and R. E. STONE.

The European corn borer.—L. CAESAR.

Studies in the physiology and cytology of Ustilago zeae and Sorosporium reilianum.—W. F. HANNA.

Mosaic diseases of plants.—G. P. CLINTON.

The relation of wound gum to fungous invasion in peach and grape.—R. S. WILLISON.

The new willow disease of Canada and New England.—G. P. CLINTON.

Black mould of onions.—J. E. MACHACEK.

Observations on the relative resistance to smut infection of some varieties of oats.—R. E. STONE.

A severe outbreak of bacterial wilt of cruciferae.—D. H. JONES

Observations on needle blight of white pine.—J. DEARNESS.

Bulb growing in Holland in its relation to disease control.—F. L. DRAYTON.

The subject is introduced with a description of the events which led up to the three months' visit to Holland in the summer of 1928 at the invitation of the Netherlands Government. An outline is given of the location of the bulb-growing districts, the research and educational institutions dealing with various phases of bulb culture, and the various associations in connection with bulb culture. The exceedingly specialized and intensive nature of successful commercial bulb culture is stressed.

The main subject matter is divided into five sections as follows: General Bulb Culture, Hyacinth, Narcissus, Tulip, and Gladiolus. In the first of these the general year-round routine is described, emphasizing the procedures related to disease control.

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